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PROCEEDINGS
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*THE EFFECT OF ANTIBIOTICS ON UREA BREAKDOWN IN MICE**

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Communicated May 4, 1953

The possibility that urea, rather than being a final end-product of protein metabolism in mammals, can be further broken down to carbon dioxide and ammonia has been suggested ever since the report by Luck[1] in 1924, of the presence of the enzyme, urease, in the gastric mucosa of a number of animals. Recently, Leifer, Roth, and Hempelmann[2] found that approximately 20% of the C^{14}-labeled urea injected intraperitoneally into mice appeared in the expiratory air as $C^{14}O_2$ in 48 hours. Kornberg and Davies[4] found that cats injected intravenously with C^{14}-labeled urea expired 1–2% of the urea as $C^{14}O_2$ in from four to five hours. When they injected N^{15}-labeled urea subcutaneously, they found that after 40 hours approximately 5% of the urea in the cat was broken down. Hastings, Langham, Carter, and Roth,[3] confirmed the results of Leifer, *et al.*, and noted that in mice, the quantity of C^{14}-urea being broken down to $C^{14}O_2$ was practically eliminated after surgical removal of the gastro-intestinal tract.

Because of these observations, a study of the distribution of urease in the gastro-intestinal tract of mice was undertaken. It soon became apparent that a significant quantity of the measured urea breakdown was being contributed by urease-positive bacteria adhering to the walls of the tissues studied, despite rather thorough washing of these tissues. To reduce bacterial contributions to tissue urease activity, a series of experiments were carried out in which groups of mice were fed a mixture of the antibiotics, penicillin, sulfaguanidine, and terramycin. The present paper deals with the effects of the antibiotic feeding on urea breakdown in mice, in vitro and in vivo.

Materials and Methods.—Young adult male mice of CF No. 1 strain from Carworth Farms were used throughout. Their average weight was 27 g. For in vitro measurements of tissue urease activity, the mice were killed by

decapitation, and the stomach, small intestine, cecum, and colon were dissected out. These organs were then cut open, halved longitudinally, and rinsed with generous quantities of sterile 0.9% NaCl until free of all adhering debris. After weighing, one piece of each pair of tissue halves was placed into each of two vessels containing 1.9 ml. of sterile $M/15$ phosphate buffer, pH 6.1, and equilibrated at 37.5°C. for 15 minutes. One-tenth ml. of 2.8M urea solution was introduced into one vessel of each pair, giving a final urea concentration of 140 mM/liter, and the CO_2 liberated in a given period of time measured manometrically in a Scholander microvolumetric respirometer.[5] It was found that under these conditions, approximately 10% of the total CO_2 produced was retained in the buffer as bicarbonate. Corrections for this quantity were included in final activity figures. Urease activity was expressed as $mm.^3$ CO_2 liberated/hour/mg. wet tissue. The vessels containing the other halves of the organs received 0.1 ml. of buffer instead of the urea, and served as controls; experimental results were corrected for any volume changes noted in the control vessels. Where fecal material was studied, fresh specimens were collected under aseptic conditions.

For measurement of urease activity in tissues incubated with isotopic urea, 0.1 ml. of a solution containing 3×10^{-6} mM urea with a total activity of 4.1×10^3 counts per minute was used. After one hour's incubation, 0.1 ml. of 6 N H_2SO_4 was injected into the vessels, and the $C^{14}O_2$ liberated collected in center wells containing NaOH. The center well contents were quantitatively removed, diluted to proper volume, and aliquots taken for precipitation of the carbonate as $BaCO_3$. Radioactivity determinations were made on weighed samples of $BaCO_3$ plated on planchets of 1.4 cm. plating diameter, in a gas flow counter in the proportional range.[6] Samples were corrected to a self-absorption of 5 mg. and referred to a $BaCO_3$ standard of 12,000 counts per minute.

Viable bacterial counts were obtained by making pour plates of serial dilutions of the organisms. Measurements of the urease activity of bacteria were made on resting cell suspensions of 24 hour cultures. Relative bacterial concentrations in suspensions were determined by optical density measurements.

For antibiotic-feeding experiments, the mice were placed in wire-bottom cages and fed ground Purina chow into which the following percentages of antibiotics had been thoroughly mixed: (1) Sulfaguanidine, 2%; (2) Crystalline Terramycin Hydrochloride, 0.1%; (3) Crystalline Procaine Penicillin, 0.3% (500,000 I. U./100 g. food). Food and water consumption were measured daily and the total antibiotic dose calculated from food consumption figures. Control mice were treated in the same manner, the antibiotics being omitted from their diets.

In vivo measurements of the breakdown of C^{14}-labeled urea were made by

placing the mice in a metabolism cage immediately after intraperitoneal injection of 0.1 ml. of a solution containing 8.3×10^{-4} mM urea with a total activity of 1×10^6 counts per minute. The CO_2 of the respiratory air was collected at intervals in alkali and radioactivity determined as already described. In experiments on fasted mice, all food was removed from the animal cages 18 hours prior to the isotopic urea injections.

Results: In Vitro Experiments.— In making measurements of the urease activities of gastro-intestinal tissues in mice, it was noted that after the tissues were removed from their vessels, and measurements continued, approximately 50% of the activity remained in the buffer solution in which the tissues had been suspended. It was found that these vessel contents contained considerable quantities of bacteria, many of them possessing the ability to convert urea to CO_2 and NH_3. If active vessel contents, includ-

TABLE 1

EFFECT OF ANTIBIOTIC FEEDING ON IN VITRO UREASE ACTIVITY OF PORTIONS OF THE GASTRO-INTESTINAL TRACT OF MICE

TISSUE OR MATERIAL	AVERAGE UREASE ACTIVITY, MM.^{3} CO_2/MG. TISSUE WET WT./HR			
	ANTIBIOTIC-FED[a]	NO. OF EXPTS.	CONTROL	NO. OF EXPTS.
Stomach	0.00	6	0.12	16
"Pre-cecum"[b]	0.00	6	0.42	7
Colon	0.00	6	0.55	21
Cecum	0.00	6	0.92	16
Feces[c]	0.00	6	2.38	16

[a] Daily antibiotic dose: Sulfaguanidine, 100 mg.; terramycin, 5 mg.; penicillin, 15 mg. (25,000 I. U.).

[b] Last 5 cm. small intestine.

[c] Viable bacterial count/mg. feces wet wt.: Control mice: 10^6; antibiotic-fed mice: <50.

ing the tissues, were homogenized in buffer and passed through a sintered glass bacterial filter, the resultant filtrate had no urease activity, showing that urease was not present in solution. In subsequent studies on the bacterial content of gastro-intestinal tract tissues and on the feces, *Aerobacter aerogenes* was one organism frequently isolated when urease activity was present. Pure cultures of this organism were found to have ureolytic activity. Bacteria killed by heat (60°C. for 15 minutes) showed the same degree of urease activity as did viable bacteria. Scrapings of washed tissues, smeared directly onto slides and gram-stained, always revealed the presence of gram-negative bacilli, gram-positive cocci, a few gram-positive bacilli, and a number of yeast cells. These were present even on the walls of the stomach. A great variation in kinds and numbers of bacteria was found in the gastro-intestinal flora of any one mouse at different times, and in different mice in similar environments. The feeding of the antibiotic

mixture, penicillin, sulfaguanidine, and terramycin, was therefore undertaken in an attempt to eliminate as many as possible of the micro-organisms of the gastro-intestinal tract.

Effectiveness of the antibiotics was followed by performing viable bacterial counts on the feces, commencing 18 hours after first including antibiotics in the food of the animals. In 24 to 48 hours after the first feeding, the fecal count dropped from an average of 10^6 bacteria per mg. feces to 50

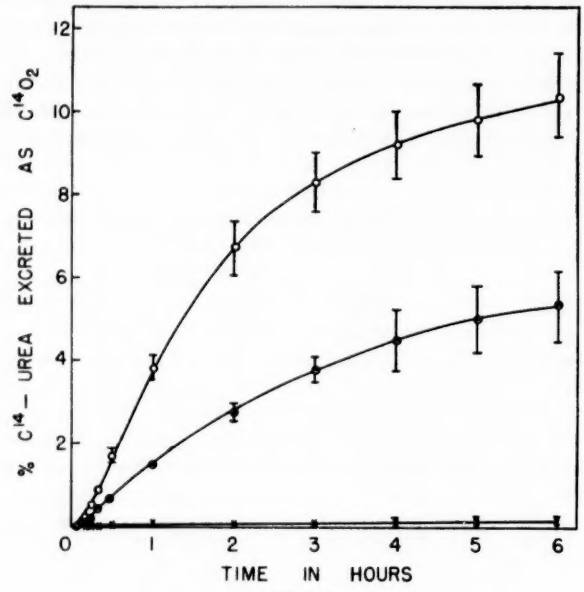

FIGURE 1

Cumulative excretion of $C^{14}O_2$ following intraperitoneal injection of C^{14}-urea.

O—designates the mean results ± the standard error of non-fasted mice. ◓—designates the mean results ± the standard error of mice fasted 18 hours. ×—designates the mean results ± the standard error of antibiotic-fed mice.

or less bacteria per mg. feces. At the time of minimal fecal bacterial count in the antibiotic-fed mice, both experimental and control mice were sacrificed and the urease activity of the gastro-intestinal tissues measured (table 1). Since the only part of the small intestine ever showing any significant urease activity was the portion 5 cm. adjacent to the cecum, it is the only portion of the small intestine included in the table.

To eliminate the possibility that urease *per se* was being inhibited either

by the antibiotics or by some product formed by the action of the antibiotics on tissues or fecal material, normal mouse gastro-intestinal tissues and feces were incubated with: (1) the antibiotic mixture, (2) a homogenate of gastro-intestinal tissues from antibiotic-fed mice, and (3) a fecal homogenate from an antibiotic-fed mouse. In a 2-hour incubation period, none of these substances had any effect on the urease activity measured. These

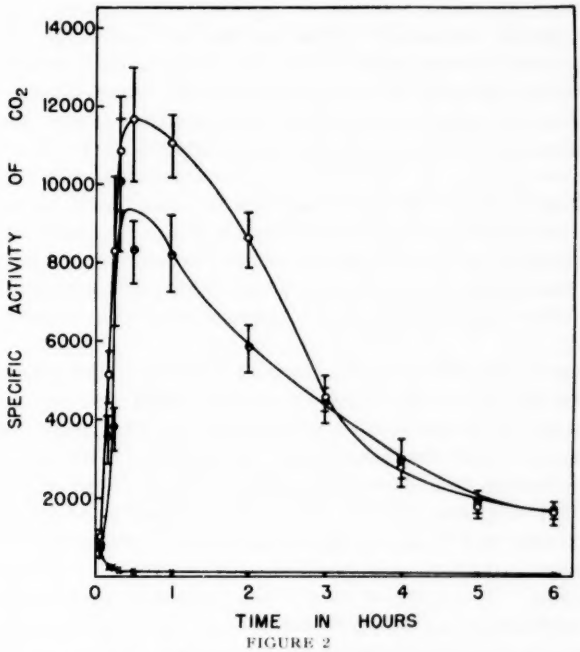

FIGURE 2

Specific activity of expired CO_2 following intraperotoneal injection of C^{14}-urea.

Specific activity = counts/min./mM carbon. ○—designates the mean results ± the standard error of non-fasted mice. ●—designates the mean results ± the standard error of mice fasted 18 hours. ×—designates the mean results of antibiotic-fed mice.

findings indicated that the antibiotics were effecting elimination of urease activity by causing reduction in bacterial numbers of the gastro-intestinal tract rather than by any direct effect on a ureolytic enzyme or enzyme system.

In Vivo Experiments.—In order to study the effect of antibiotic feeding on in vivo urea breakdown, mice were injected intraperitoneally with C^{14}-

labeled urea, and their rate of breakdown of the urea measured. Immediately upon removal from the metabolism cage, the animals were put on diets containing the antibiotic mixture, and when the minimum bacterial fecal count was reached, their rate of urea breakdown, as measured by $C^{14}O_2$ excretion, was again determined. Figure 1 shows the cumulative excretion of the $C^{14}O_2$ by normal, fasted, and antibiotic-fed mice. From this data alone, it would appear that fasted mice have considerably reduced urea breakdown, whereas urea breakdown by antibiotic-fed mice has been almost completely eliminated. However, when the specific activity of the $C^{14}O_2$ excreted is plotted against time (Fig. 2), it can be seen that there is little difference between that of normal and fasted mice. The antibiotic fed mice showed an almost negligible urea breakdown, even though the total quantity of CO_2 they expired in a given period of time was the same as that of normal mice. In experiments on two mice, the inclusion of penicillin alone in the food in the same dosage level as that used in the mixture of antibiotics, seemed to be equally as effective as the mixture in eliminating urea breakdown in vivo. This was not the dose for either sulfaguanidine alone or terramycin alone. It is presumed, therefore, that penicillin, in the concentrations used, was the most important constituent of the antibiotic mixture.

After in vivo experiments were completed, the mice were sacrificed, and the urease activity of their gastro-intestinal tissues measured using C^{14}-labeled urea. With this procedure, no urease activity could be detected in the tissues of the antibiotic-fed mice. It was found that mice taken off antibiotic-feeding could regain their ability to break down isotopic urea in vivo within nine days, and that this return of ureolytic activity coincided with increased fecal flora and the appearance of urease-positive organisms in the feces.

Discussion.—The results of these experiments seem to indicate that the mouse possesses many cells with ureolytic activity, but that these cells, rather than being integral parts of the mouse tissue, are bacteria present in the contents and growing and adhering to the walls of the gastro-intestinal tract. In the course of incubation and shaking of tissues for urease determinations, many of these bacteria become dislodged from the tissues and remain in the buffer solution after the tissues are removed. Residual activity in the vessel contents is due to these dislodged bacteria.

In table 1, it can be noted that colon activities are lower than those of the cecum. Yet the colon contains feces with a very high urease activity. This apparent contradiction is explained by the fact that the fecal material in the cecum is fairly loose and mucoid, and adheres tenaciously to the tissue walls despite vigorous washing of the surface. On the other hand, the fecal material in the colon is usually present in well-formed packets which are readily removed, leaving the tissue relatively free of adhering debris.

Thus, the cecal tissues carry along with them relatively large quantities of bacteria, giving the tissue an apparently high urease activity.

Normally, large quantities of bacteria are swept out of the gastro-intestinal tract in the feces. Due to the constant multiplication of gut organisms, the total number present in the tract remains fairly high. With the feeding of antibiotics, however, multiplication of the organisms is inhibited. The rate of fecal formation and elimination in mice is high, and it is not surprising that the quantity of gut bacteria is considerably reduced in as little as 24–48 hours after commencement of antibiotic feeding. Both in vitro urease activity and in vivo urea breakdown are eliminated simultaneously by the feeding of the antibiotic mixture. Calculations based on the apparent urease activities of the gastro-intestinal tissues and their contents show that in vivo urea breakdown can be accounted for by the urease activity in the gastro-intestinal tract.

Measurements of urease activity were made on the basis of the carbon dioxide produced as a result of urea breakdown. However, the simultaneous production of 2 moles of ammonia for each mole of urea hydrolyzed cannot be overlooked. Ammonia has long been believed to be a substance toxic to most living cells. So-called ammonia poisoning, presumably originating as the result of breakdown of body urea, can be brought on by injection of jack-bean urease.[7] Also, death from ammonia poisoning frequently follows the administration of large quantities of urea to rabbits.[8] The studies presented here indicate that the gastro-intestinal flora can be a potential source of ammonia, and that these levels might become dangerously high in those abnormal conditions characterized by uremia or great reduction in the capacity of the liver to synthesize urea. Use of antibiotics as a means of lessening ammonia production in the gastro-intestinal tract might therefore prove to be beneficial in clinical situations associated with severe liver damage.

Summary.—In a study of the distribution of urease in the gastro-intestinal tract of mice, it was found that the feeding of a mixture of sulfaguanidine, terramycin, and penicillin completely abolished all significant urease activity. Evidence is presented which suggests that urea catabolism in mice and, by inference in other mammals, is due to the presence in the gastro-intestinal tract of bacteria possessing urease activity. Some implications of this potential ammonia source in the animal body are discussed.

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PATH OF ORNITHINE SYNTHESIS IN ESCHERICHIA COLI

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Communicated by F. Lipmann, June 8, 1953

The mode of ornithine biosynthesis has been investigated in a number of organisms, including fungi[1-3] and mammals;[4-10] however, it has not been possible to establish with certainty a pathway of ornithine formation in any one species. These investigations were complicated by the close relationship which has been found to exist between the metabolism of ornithine and that of proline;[1-10] it has been suggested that the paths of ornithine and proline formation may be linked by glutamic γ-semialdehyde as a common intermediate.[2, 9, 10] In *Escherichia coli*, glutamic γ-semialdehyde has been shown to be a precursor of proline,[11] but does not appear to participate directly in ornithine synthesis. It was therefore of interest to examine the ornithine pathway in this organism; the results of such an investigation form the subject of this report.

The experiments described here have been conducted with the aid of mutant strains of *E. coli* which, characteristically, accumulate metabolites as a result of a block in ornithine synthesis. Evidence that these metabolites are intermediates in ornithine formation is provided not only by their accumulation, but also by a study of enzymes contained in extracts of the parental wild-type strain. The present findings indicate that in *E. coli* ornithine arises from glutamic acid via N-acetylglutamic acid, N-acetyl glutamic γ-semialdehyde, and N^{α}-acetylornithine as intermediates (see Fig. 1).

N^{α}-Acetylornithine.—Culture filtrates of an ornithine-requiring mutant strain (160-37) derived from *E. coli*, ATCC No. 9637, have previously been shown to contain N^{α}-acetylornithine.[12, 13] The accumulation of this compound as a consequence of an impairment of ornithine synthesis indicated that N^{α}-acetylornithine is a precursor of ornithine. This indication was

supported by the finding that an enzyme capable of converting N^{α}-acetylornithine to ornithine could be detected in extracts of wild-type *E. coli*, but not of mutant strain 160-37.[12] It has now been found that the activity of this enzyme is enhanced by the cobaltous ion.† In this respect the enzyme resembles another acylase[14] and some peptidases.[15] Partial purification has not revealed any other cofactor requirements; the cleavage of N^{α}-acetylornithine therefore appears to be a hydrolytic reaction.

N-Acetylglutamic γ-Semialdehyde.—The fact that strain 160-37 has an ornithine requirement indicates that the accumulated N^{α}-acetylornithine does not arise from the acetylation of ornithine. A search for a possible precursor of N^{α}-acetylornithine has revealed that culture filtrates of strain 160-37 also contain a substance identified as N-acetylglutamic γ-semialdehyde, presumably related to the L-series of amino acids. For the isolation of this substance, a culture of strain 160-37 was grown with aeration in "Medium B"[11] at 30°C. for two days and then filtered. The pH of the resulting filtrate was adjusted to 8.5 and inorganic anions were precipitated with a slight excess of aqueous barium acetate. The precipitated barium salts were filtered off and the solution obtained was freed of cations by passage through a column of Amberlite IR-120 (acid form). From the resulting effluent, water, acetic acid, and other volatile substances were removed in vacuo. The residue was dissolved in water and the resulting solution was extracted exhaustively with ether. The material remaining in the aqueous layer was partitioned between *n*-butanol and water. The contents of the butanol phase were chromatographed on silicic acid (Mallinckrodt) and upon elution with water-saturated ethyl acetate, N-acetylglutamic γ-semialdehyde (about 10 mg. per liter of original culture filtrate) was isolated as a colorless, viscous liquid. This aldehyde reacted with 2,4-dinitrophenylhydrazine to give a yellow, crystalline derivative melting, after recrystallization from absolute methanol, at 208°C. (*Analysis.*[16] Calculated for the dinitrophenylhydrazone, $C_{13}H_{18}O_7N_8$: C, 44.2; H, 4.3; N, 19.8. Found: C, 44.0; H, 4.3; N, 20.1.) The original aldehyde could be regenerated by treatment of this derivative with benzaldehyde in acidified ethanol. An acid hydrolysate of N-acetylglutamic γ-semialdehyde was found to contain acetic acid; after neutralization, the hydrolysate produced a yellow color with *o*-aminobenzaldehyde and satisfied the growth requirement of mutant strain 55-25, but not of mutant strain 55-1 of *E. coli* (see Fig. 1 for the site of the blocks in these strains). This color reaction and growth response are characteristic of Δ^1-pyrroline-5-carboxylic acid, the cyclized form of glutamic γ-semialdehyde.[11] The reaction with *o*-aminobenzaldehyde after acid hydrolysis was used to follow the progress of purification of the N-acetylglutamic γ-semialdehyde.

From the excretion of N-acetylglutamic γ-semialdehyde by strain 160-37 and from the structural similarity of this compound to N^{α}-acetylornithine

it was inferred that the aldehyde is a precursor of N^{α}-acetylornithine and hence of ornithine. This inference received support through the finding that dialyzed cell-free extracts of strain 160-37 and of the corresponding wild-type strain contain an enzyme which, in the presence of glutamate, converts N-acetylglutamic γ-semialdehyde to N^{α}-acetylornithine. This enzyme thus appears to be a transaminase; its activity could be stimulated by addition of pyridoxal phosphate.† Other instances of transamination involving an aldehyde group have recently been described.[3, 17, 18]

N-Acetylglutamic Acid.—The chemical structure of N-acetylglutamic γ-semialdehyde suggested that this compound might be derived from glutamic acid. This view is consistent with the results of Abelson, Bolton, and Aldous[19, 20] on the incorporation of labeled carbon dioxide into amino acids of *E. coli*, including glutamic acid and arginine, which is known to be derived from ornithine. The recent finding by Maas, Novelli, and Lipmann[21] that extracts of *E. coli* contain an enzyme which catalyzes the N-acetylation of glutamic acid makes it reasonable to suppose that N-acetylglutamic acid is a precursor of N-acetylglutamic γ-semialdehyde. This aldehyde may thus arise from glutamic acid by an acetylation step followed by a reduction step. The reverse of this reaction sequence, i.e., reduction followed by acetylation, has now been shown to be unlikely. This negative finding lends a measure of support to the supposed participation of N-acetylglutamic acid in ornithine synthesis.

The evidence against the possibility that N-acetylglutamic γ-semialdehyde is formed from glutamic acid by reduction to glutamic γ-semialdehyde followed by N-acetylation has been obtained from a comparison of the excretion of N^{α}-acetylornithine by strain 160-37 with that by two double mutant strains derived from strain 160-37. These double mutant strains had, in addition to the block in ornithine synthesis, a block in the proline pathway; one of the strains (160-37D1) was blocked before glutamic γ-semialdehyde, and the other (160-37D2) was blocked between glutamic γ-semialdehyde and proline (see Fig. 1). When grown on minimal medium supplemented with 4μg. per ml. L-proline and 5μg. per ml. L-ornithine monohydrochloride, these double mutant strains both excreted 30μg. per ml. N^{α}-acetylornithine; exactly the same amount of this substance was excreted by strain 160-37 when grown on a supplement of 5 μg. per ml. L-ornithine monohydrochloride without added proline, but under otherwise unchanged cultural conditions. Since one of the double mutant strains was blocked in the synthesis of glutamic γ-semialdehyde, the availability of this compound does not appear to affect the excretion of N^{α}-acetylornithine. Consequently, glutamic γ-semialdehyde probably is not a precursor of N^{α}-acetylornithine in *E. coli*.

Discussion.—The present findings taken together with those of Maas, Novelli, and Lipmann[21] and of Abelson, Bolton, and Aldous[19, 20] support

the path of ornithine synthesis in *E. coli* shown in figure 1. The existence of mutant 160-37 in which the impairmant of a presumably single biochemical function[22, 23] has given rise to a requirement for ornithine, suggests that the path of ornithine synthesis described here is the only major one in *E. coli*.

It is noteworthy that mycelial extracts of Neurospora have recently been reported to catalyze the transamination of glutamic γ-semialdehyde to

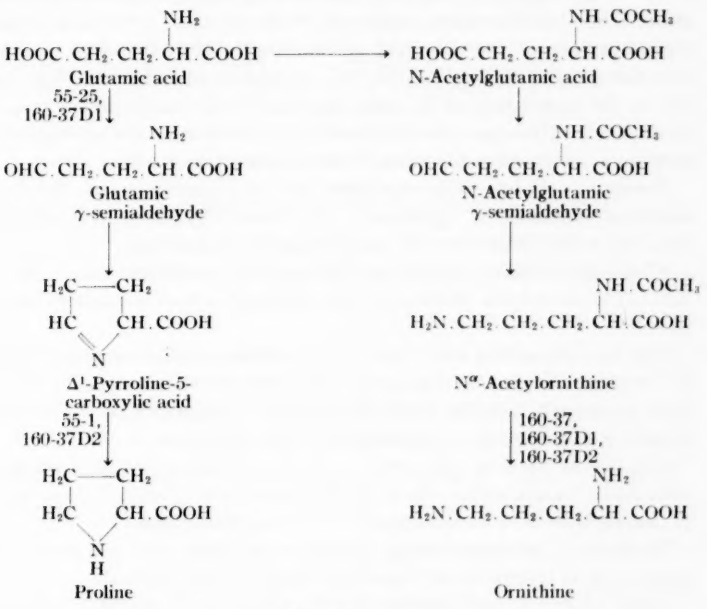

FIGURE 1

Paths of ornithine and proline synthesis in *Escherichia coli*. The sites of blocks in the mutants used, inferred from growth response and precursor accumulation, are indicated by the location of the respective strain numbers. Strains 160-37D1 and 160-37D2 each have two separately induced blocks, as shown.

ornithine.[3] This finding is in harmony with the formulation of the ornithine pathway proposed for this organism by previous authors.[2] On the other hand, the additional finding that ornithine-requiring mutant strains of *N. crassa* have approximately as much transaminase activity as the corresponding wild type led to the suggestion that perhaps another ornithine pathway might be operative in this species.[3] If so, it is nevertheless unlikely that the ornithine path reported here for *E. coli* is a major one in

Neurospora, since N^{α}-acetylornithine does not satisfy the growth requirement of two different mutant strains of *N. crassa*, one of which responds specifically to ornithine, whereas the other responds alternatively to ornithine, Δ^1-pyrroline-5-carboxylic acid, or proline.[24]

The acetylated ornithine precursors encountered in the present studies recall the early suggestion of Knoop[25] that acetylamino acids may be intermediates in the conversion of α-keto acids to the corresponding amino acids. This suggestion has stimulated much fruitful work on the metabolism of acetylamino acids, although it has not been possible to establish the occurrence of the proposed mechanism.[26] It is therefore of interest that the present findings permit the assignment of a definite biosynthetic role to the acetylation of an amino acid and the deacetylation of an acetylamino acid. However, the acetylated intermediates in the ornithine pathway are not formed in the manner contemplated by Knoop.

Summary.—It has been concluded that in *Escherichia coli*, the path of ornithine synthesis is: glutamic acid→N-acetylglutamic acid→N-acetylglutamic γ-semialdehyde→N^{α}-acetylornithine→ornithine.

N^{α}-acetylornithine and N-acetylglutamic γ-semialdehyde have been isolated from culture filtrates of an ornithine-requiring mutant strain of *E. coli*.

Cell-free extracts of wild-type *E. coli* contain a cobaltous ion-stimulated deacetylase capable of converting N^{α}-acetylornithine to ornithine. No such enzymatic activity could be detected in extracts of the ornithine-requiring strain which accumulates N^{α}-acetylornithine.

Cell-free extracts of wild-type *E. coli* also contain a pyridoxal phosphate-stimulated transaminase which, in the presence of glutamate, can convert N-acetylglutamic γ-semialdehyde to N^{α}-acetylornithine.

Evidence is presented which points to glutamic acid and N-acetylglutamic acid as precursors of N-acetylglutamic γ-semialdehyde.

Some aspects of the comparative biochemistry of ornithine and proline formation have been discussed.

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* Damon Runyon Memorial Fellow.

† Details of the enzymatic studies will be published elsewhere.

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*PULCHERRIMIN, THE PIGMENT OF CANDIDA PULCHERRIMA**

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In 1901 Lindner[1] described an, at that time, unknown yeast species to which he gave the name *Torula pulcherrima*, the specific name being connected with the beautiful aspect of its microscopic preparations when the organism had been grown under special conditions: strictly spherical cells of uniform size, each containing a large, highly refractile globule apparently rich in fat. Since then various properties of this yeast, which in present-day taxonomy is known as *Candida pulcherrima* (Lindner) Windisch, have been studied by several authors. In this article we shall restrict ourselves to the property of the organism to produce a red pigment under certain cultural conditions.

The first important observations on the conditions determining pigment production were made by Beijerinck.[2] In 1918 he established that this production was markedly increased by the addition of a certain amount of an iron salt to the media normally used for yeast cultivation. More-

over, it was found that on plating a culture on such a medium the ability to produce pigment was not shared by all colonies. Often the greater part of these remained perfectly white, while others had assumed a deep maroon-red color. We may conclude from this that pigment production is a property of a mutant form which can appropriately be designated as the "red mutant." Beijerinck further reported that the pigment in question was fully different from the carotenoid pigments present in the normal red yeasts belonging to the genera Rhodotorula and Sporobolomyces. He found that the pigment was only very slightly soluble in water—apparently in a colloidal state—and insoluble in all organic solvents tested. Beijerinck established, however, that the pigment readily dissolved in alkali and could be reprecipitated from the solution by addition of acid. The pigment was remarkably stable, and even resisted boiling with dilute sulphuric acid.

Interesting observations on the appearance of the red mutant of *Candida pulcherrima* and its reversal to the colorless form have since been published by Punkari and Henrici,[3, 4] Porchet,[5] Castelli,[6] and Roberts.[7, 8] These studies shall not be discussed in any detail here; a few remarks will suffice. The two last cited authors fully confirmed Beijerinck's observation that pigment production is directly connected with the iron content of the medium, while Roberts in her second publication made a thorough study of the factors determining pigment production. Important is her observation that all strongly pigmented cells proved to be non-viable, showing that pigment production is a more or less pathological process. In a way this supports Beijerinck's hypothesis that the production of pigment is a defense reaction of the organism against a harmful effect of an excess of iron present in the medium.

Origin and Maintenance of the Strain Used in the Investigation.—A rather large number of strains of *Candida pulcherrima* present in the collection of the "Centraalbureau voor Schimmelcultures" were tested on their ability to form strongly pigmented mutants. Strain Nr. 35.2.8, originally received under the name of *Cryptococcus interdigitalis* from Pollacci and Nannizzi, was selected as being especially satisfactory. It proved possible to maintain the red mutant of this strain in a practically pure state by subculturing it at regular intervals on a medium containing: 4 g. saccharose, 2 g. Bacto-peptone, 0.2 g. KH_2PO_4, 0.05 g. $MgSO_4 \cdot 7H_2O$, 1 ml. yeast autolysate, 2 g. agar, 0.05 g. ferric ammonium citrate, 100 ml. tap water. Streak cultures on this medium had a deep maroon color. From time to time a reversal to the white form was noticeable. If so, plating followed by selection of a suitable red colony sufficed to restore the situation.

Large-Scale Production of the Red Yeast.—In the earlier part of the investigation large quantities of the yeast were grown on a suitable agar

medium in Kolle flasks which were preferred to Petri dishes in view of airborne contaminations. This was done because it was found that in ordinary liquid media pigmentation was only feeble, even under conditions of plenty aeration. Since cultivation in a liquid medium seemed to offer many advantages, a more or less systematic search for more suitable media and optimal conditions for cultivation was made. It was finally found that an excellent result, as far as pigmentation was concerned, could be obtained by cultivating the red mutant in a medium consisting of 2% corn steep liquor, 4% saccharose, and 0.036% ferric ammonium citrate, provided that a sufficient aeration was applied by continuous agitation by a rotary shaker. It appeared, however, that pigmentation was highly dependent on the degree of aeration; both the amount of liquid in the flask and the shaking intensity greatly influenced the result. The procedure finally adopted was to bring 100 ml. culture medium in 500 ml. glass flasks, cultivating at 30°C. in a shaking device with 300 rev./min. The average yield was about 50 g. wet yeast (±75% moisture) per liter medium.

Preparation of the Pigment.—Beijerinck's observation that the pigment was insoluble in all organic solvents tested by him was confirmed and extended over some 30 different solvents. Under these conditions the only way to extract the pigment from the yeast cells seemed to be the use of caustic alkali and reprecipitation of the pigment by acidification. This led to the following procedure.

The yeast is collected from the medium by centrifugation, and then twice washed with methanol (500 ml. per 100 g. yeast). After recollecting the yeast by centrifugation, the red sediment was treated with alkaline methanol (per 100 g. yeast: 50 ml. of a 50% aqueous solution of KOH to which 450 ml. methanol had been added). The pigment readily disappears from the cell. After renewed centrifugation, and filtration of the supernatant through a glass filter, a clear yellowish brown solution is obtained. After addition of 1 ml. of a 5% solution of $FeCl_3$ to 1 l. of the alkaline solution the latter is acidified by adding a sufficient amount of concentrated hydrochloric acid to attain a pH = 1.0. This leads to a reprecipitation of the red pigment which can be collected on a glass filter. The pigment is successively washed with water, methanol, ether, diluted hydrochloric acid, and distilled water. The pigment is then resuspended, boiled with methanol, very diluted hydrochloric acid and, finally, with distilled water. After refiltering and repeated washing with water, ethanol, and ether the pigment is dried *in vacuo* at a temperature of 80°C.

From 250 to 300 mg. of an amorphous deep maroon to bordeaux-red powder is obtained per 100 g. wet yeast. This pigment will be indicated as pulcherrimin.

Properties of Pulcherrimin.—It is clear that the described procedure of

isolation suffers from serious shortcomings. It is quite unusual to isolate a biological principle with strong alkali, and no guarantee can be given as to the purity of the amorphous product obtained. In view of the properties of the pigment no better method could be devised. Moreover, it appeared that the elementary composition of preparations from various yeast batches showed only minor variations, suggesting that only slight quantities of impurities could be present.

The elementary analysis of one of the preparations yielded: 48.2% C, 6.5% H, and 9.2% N. The great surprise was the high amount of ash obtained which proved to be pure Fe_2O_3, indicating an iron content of the pigment of not less than 12.4%. Since the pigment had been precipitated in a medium with a pH = 1.0 the presence of inorganic iron impurities can be ruled out, and the conclusion seems warranted that pulcherrimin is an example of a biological compound with an exceptionally high iron content.

A second remarkable property of pulcherrimin proved to be its quite unusual resistance to relatively strong acids. In an attempt to bring about an acid hydrolysis some pigment was boiled for several hours with a mixture of 50% concentrated hydrochloric acid and 50% ethanol. Even after 6 to 10 hours boiling at least part of the pigment remained undissolved. The unexpected observation was made that the iron content of various preparations had not been reduced by this operation, but to the contrary had increased, in all cases approaching the value of 12.7%. The rather drastic procedure described seems, therefore, even suitable to attain a final purification of the pigment. It will be needless to remark that this behavior is quite abnormal for an organic iron compound.

Notwithstanding this obviously very high resistance against acid hydrolysis, the resulting liquid was tested for the presence of amino acids by paper chromatography. The presence of a non-negligible quantity of leucine could be established with certainty; apart from an indication for the presence of some aspartic acid other amino acids seemed to be present either only in very slight traces, or not at all. This result seems to exclude the possibility of pulcherrimin being a protein.

An unsuccessful attempt to determine a melting point of pulcherrimin led to the observation that at approximately 150°C. the pigment begins to decompose, and that after some time a crystalline product accumulates at the colder part of the tube. More systematic experiments on pyrolysis, in which a mixture of pulcherrimin and zinc dust was gradually heated in vacuum, were then made. At 190°C. a white sublimate is formed, and at 220° crystalline needles are clearly apparent. Some oil drops are also formed, and small quantities of water and also ammonia could be detected in the vapor. The white needles could be recrystallized from a methanolic solution, and showed a triple point of 294°C. (corr.).

Micro-elementary analysis yielded: 65.1% C, 8.3% H, and 12.8% N. Calculated for C_6H_9NO: 64.9, 8.2, 12.6.

Although no molecular weight could be determined according to Rast's micromethod, indications were obtained suggesting a doubling of the empirical formula to $C_{12}H_{18}N_2O_2$.

It happened that at that time our attention was drawn to the studies of

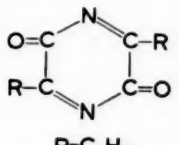

FIGURE 1
Product of pyrolysis of pulcherrimin

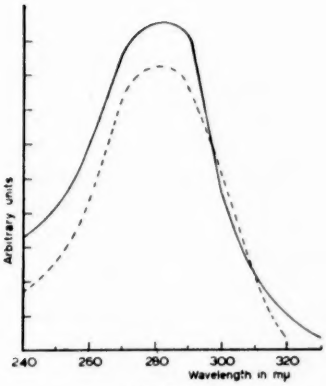

FIGURE 2
Ultra-violet absorption spectrum of:
——pyrolysis product of pulcherrimin
– – Dutcher's compound $C_{12}H_{18}N_2O_2$

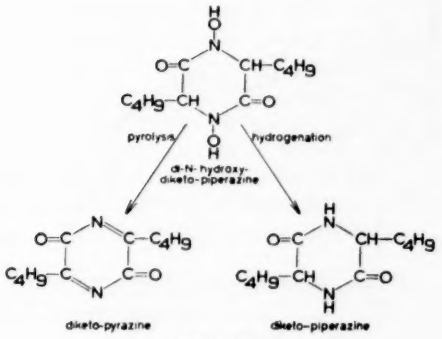

FIGURE 3

Dutcher[9, 10] on the constitution of aspergillic acid. It struck us that this investigator had obtained from the mother liquor of bromoaspergillic acid a colorless neutral product of high melting point also having the formula $C_{12}H_{18}N_2O_2$. To this compound he assigned the constitution given in figure 1. It was found that there was a close—although not complete—agreement in properties of this product and those of our pyrolytic product,

as is shown, among other evidence, by the ultra-violet spectra of the two compounds (Fig. 2).

On the ground of these similarities it seems reasonable to attribute also to our product a diketo-pyrazine nucleus $C_4N_2O_2$, leaving C_8H_{18} for two aliphatic side chains. The formula given in figure 1 could then also hold for the structure of the product of pyrolysis, although there may be structural differences in the side-chains R.

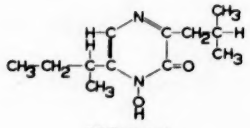

FIGURE 4
Aspergillic acid (after Dutcher)

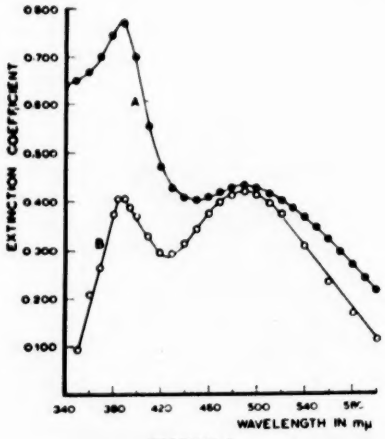

FIGURE 5
Absorption spectrum of: *A*, pulcherrimin; *B*, ferric complex of aspergillic acid

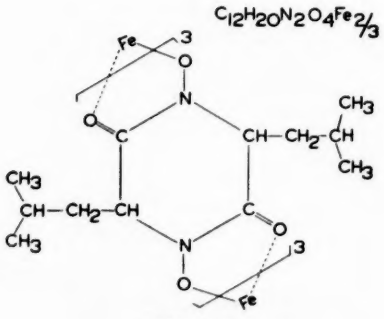

FIGURE 6
Iron complex of di-N-hydroxy 3,6-di-sec-butyl-2,5-diketo-piperazine

This idea has now gained in probability by experiments on reductive pyrolysis of pulcherrimin. In these experiments a mixture of pulcherrimin and zinc dust was suspended in glacial acetic acid, after which a solution of palladium chloride in the same solvent was added. The palladium is finely precipitated as metal and thus intimately mixed with the pulcherrimin and zinc dust. After filtration the mixture is slowly heated in a hydrogen current. Again a fine needle-shaped sublimate was formed

which, however, differed in various respects from the normal product of pyrolysis. Although until now only a small quantity has been obtained, comparison of this product with a synthetic preparation of leucine anhydride is strongly in favor of their identity. A chromatographic analysis of the solution obtained by boiling the product with concentrated hydrochloric acid gave definite proof for the presence of leucine, the synthetic preparation yielding the same result.

All the preceding evidence points to the presence in the pigment of a nucleus which on pyrolysis yields a diketo-pyrazine and on hydrogenation a diketo-piperazine (Fig. 3). These results seem to imply at least some configurational relationship with aspergillic acid (Fig. 4). The latter compound is known to give a beautiful red color with ferric salts. Owing to the courtesy of Dr. James D. Dutcher of the Squibb Institute for Medical Research, New Brunswick, N. J., who kindly put a pure preparation of aspergillic acid at our disposal, it was possible to determine the absorption spectrum of the ferric complex of this acid. In figure 5 this spectrum is shown together with the absorption spectrum of an aqueous colloidal solution of pulcherrimin.

As will be seen the agreement between the two spectra is most striking; the two absorption maxima at about 390 and 490 mμ coincide in an almost perfect way. The investigations of Dutcher, supplemented by those of Dunn, *et al.*,[11] have elucidated the configuration of aspergillic acid; they leave no doubt that the formation of the red iron complex is directly connected with the hydroxamic grouping occurring in the molecule. It is well known that several aliphatic hydroxamic acids also yield intensively red iron complexes in which one atom of iron is linked with three identical ligands, these complexes all having an absorption maximum in the vicinity of 500 mμ (Chantrenne[12]). For the ferric complex of butyrohydroxamic acid we could confirm this statement; the absorption maximum coincided closely with that of pulcherrimin.

The foregoing strongly suggests that also in pulcherrimin the ferric iron binding ligands contain a hydroxamic acid group present in a ring system, and that this configuration will be responsible for the red color.† This view was confirmed by the demonstration of hydroxylamine as such in the liquid obtained by prolonged boiling of pulcherrimin in concentrated hydrochloric acid. This was demonstrated by oxidation of the hydroxylamine to nitrous acid, subsequent diazotization of sulphanilic acid and coupling the resulting compound with α-naphthylamine, and also directly by the formation of nickel dimethylglyoxime.

Taking into account that in the pigment the molecular ratio of N and O approaches 1 : 2, and also the fact that hydrogenation of pulcherrimin leads to a diketo-piperazine, a symmetrical configuration of the pulcherrimin-nucleus seems probable. This suggests the presence of two hydroxamic

groups in the said nucleus, as indicated already in figure 3. This assumption will at once explain the insolubility and high molecular weight of pulcherrimin as contrasted with the solubility and low molecular weight of the ferric complexes of the monohydroxamic acids. For it will be clear that the two-sided binding of the pigment nucleus with iron, as indicated in figures 6 and 7 opens the possibility of forming complexes in which the Fe atoms act as a cement leading to the formation of a network of an indefinite number of pigment nuclei.

This would imply a structure of pulcherrimin as indicated in the schematic pattern given in figure 8. Accepting the pigment unit to be, indeed, an iron chelate of di-N-hydroxy 3,6-di-sec-butyl-2,5-diketo piperazine

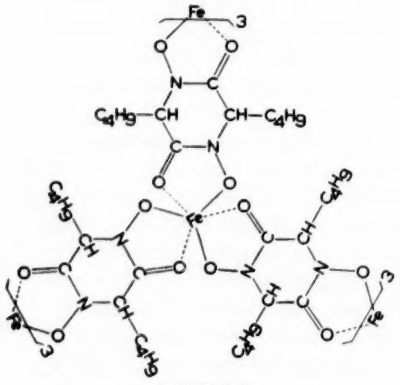

FIGURE 7
Pulcherrimin: unit configuration

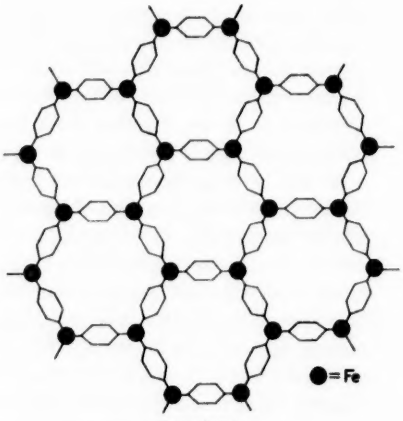

FIGURE 8
Pulcherrimin: molecular pattern

(Fig. 6), the empirical composition of the unit and at the same time that of the pigment, should be: $C_{12}H_{20}N_2O_4Fe^{2}/_{3}$.

It is now most gratifying that the elementary composition of one of the apparently purest preparations of pulcherrimin is in close agreement with the values calculated for the proposed structure, as shown in table 1.

Although the authors are fully aware of the fragmentary nature of the argumentation given, and therefore of the tentative character of the configuration of pulcherrimin as presented above, the various and very divergent observations made fit so well in the total picture that it seems likely that its main features will not be altered by subsequent research. Synthetic work, indispensable for a definite proof, has been started in the Biochemical Department of the Institute of Technology at Delft.

Although microscopic observation seems to indicate that in highly pigmented cells the pigment is encrusted in the cell wall, partly covering its outside, it is quite possible that initially pulcherrimin is in some way linked with some protein component of the cell. With a view to the primitive character of the isolation procedure of the always amorphous pigment, such a linking may explain why preparations with a somewhat lower iron content are obtained from certain batches of yeast. This content can then, however, be raised to a limit of 12.7% by boiling with acid. Whether this operation only removes impurities (proteins?), or whether a part of the pigment as it naturally occurs is split off, leaving the chromatophoric part, must be left undecided.

In any case a very high degree of pigmentation of the cell mass has been found to be essential for obtaining in the simple way described a pulcherrimin preparation of satisfactory purity.

Conclusion with Regard to the Chemical Nature of Pulcherrimin.—Among biological compounds pulcherrimin takes a special place because of its high iron content. It is true that in this respect it is surpassed by ferritin, the brown pigment occurring in the spleen, bone marrow, and liver, first ob-

TABLE 1

EMPIRICAL CONSTITUTION OF PULCHERRIMIN

$C_{12}H_{20}N_2O_4Fe_{2/3}$

	EXPERIMENTAL	THEORETICAL
C, %	48.2	49.1
H, %	6.5	6.8
N, %	9.2	9.8
O, %	23.5	21.9
Fe, %	12.4	12.7

tained in a crystalline state by Laufberger,[13] which contains about 20% Fe. Here, however, the iron can easily be removed, leaving a protein skeleton, the so-called apoferritin, of a molecular weight of 460,000, and which on hydrolysis yields at least 14 different amino acids. Obviously there is no relationship between pulcherrimin and ferritin, and the same can be said for pulcherrimin and the hemoglobins.

There may be a closer relationship with the interesting iron-containing pigment recently isolated from the rust fungus *Ustilago sphaerogena* by Neilands[14, 15] and described by him under the name of ferrichrome. On hydrolysis ferrichrome seems to yield only two organic compounds: glycine and a second component behaving as a dibasic amino acid. Ferrichrome, however, only contains 7.35% Fe, has the low molecular weight of 700–800, and is soluble in various organic solvents from which it can be obtained in the crystalline state.

On the Occurrence of Pulcherrimin in Nature.—The interest in pulcherrimin as a biological compound is greatly enhanced by the fact that its oc-

currence is apparently not at all restricted to *Candida pulcherrima*. Beijerinck had already established in 1918 that several other yeast species reacted on an addition of a ferric salt to their media by producing a red pigment, although usually to a much smaller degree than in the case of *C. pulcherrima*. We have been able to confirm this statement, and can add that the same holds for various strains of the spore-forming bacterium *Bacillus subtilis*. To this comes the observation of Cutts and Rainbow[16, 17] that several very common yeasts of the bakers and brewers type, which on normal media grow perfectly colorless, produce a red pigment on media deficient in biotin. Preliminary observations indicate that here also the pigment is pulcherrimin or a closely related substance. The same seems to hold for the red pigment spontaneously produced by haploid mutants of *Saccharomyces cerevisiae* as isolated by Lindegren and more closely studied by Ephrussi, *et al.*[18, 19] But also in these haploid forms pigmentation is increased by a deficiency in various growth factors, as was shown by Slonimski and Tavlitzki.[20] Although the solubility of the pigment in isoamyl alcohol disproves its identity with pulcherrimin, the close agreement of the absorption spectra of the two compounds points also to an iron complex of some compound with a hydroxamic grouping.

Apparently the production of pulcherrimin is, in the cases considered, connected with some interference with the amino acid metabolism of the cells; but we shall refrain from further discussion here.

* Part of the work reported in this paper has previously been published in the doctorate thesis of J. P. van der Walt entitled: "On the yeast *Candida pulcherrima* and its pigment" ('s-Gravenhage, 1952). In this publication more ample information regarding the yeast in question can be found.

† *Note added in proof:* Dr. Dean Burk was so kind as to direct our attention to the studies of A. L. Schade and collaborators on the iron-binding proteins siderophilin and conalbumin, for which also a hydroxamic acid grouping has been assumed responsible for the iron binding (cf. Fiala, S., and Burk, D., *Arch. Biochem.*, **20,** 172 (1949)).

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*TAXONOMIC SIGNIFICANCE OF ROOT-HAIR DEVELOPMENT IN THE GRAMINEAE**

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Communicated by E. W. Sinnott, April 29, 1953

That the genera of the large and complex family Gramineae fall rather naturally into two groups, or subfamilies, has long been recognized. In general we may say that in one group (the Panicoideae) the spikelets are dorsally compressed, they disarticulate *below* the glumes, and there is a tendency for aborted or rudimentary flowers to be borne *below* the fertile ones. In the other group (the Festucoideae), on the other hand, the spikelets are usually laterally compressed, they disarticulate *above* the glumes, and aborted or rudimentary flowers are borne *above* the fertile ones. The characteristics noted above are all of a gross morphological nature, but it is now known that there are also fundamental cytological and anatomical differences among representatives of grass genera and that these likewise fall into two groups which correspond, in the majority of cases, to those based upon gross morphology. For detailed discussions of the value of cytology and anatomy in the classification of the Gramineae, the reader is referred to the comprehensive works of Avdulov[1] and of Prat.[2]

Studies of sections of mature seeds have revealed that the embryos of members of this family are also of two distinct types. This was first pointed out by van Tieghem[3] in 1897, and has been verified recently by the senior author of the present paper. Moreover, there is a close correlation between the type of embryo and cytological and anatomical characters. While a classification based upon these criteria would correspond in many respects with one in which only the traditional gross morphological features were used, some important differences would be evident. One of these involves the tribe Chlorideae which long had been considered a member of the subfamily Festucoideae, but whose members show closer affinities to panicoid genera in cytological (Avdulov), anatomical (Prat), and embryo (Reeder[4]) characters. Within the tribes Festuceae and Agrostideae also some genera appear to be misplaced when they are examined cytologically and anatomically. Examples in the former tribe are Eragros-

tis and allied genera, and in the latter Muhlenbergia and Sporobolus. Thus it appears that superficial morphological similarity does not necessarily indicate close relationship, and if a natural classification of the Gramineae is to be achieved, more weight must be placed upon cytological and anatomical evidence than upon those gross morphological characters which have been traditionally used in the taxonomy of the group.

Recently there has come to our attention additional information which further emphasizes the fundamental differences between representatives of the two subfamilies. This evidence, moreover, shows a close correlation with results from cytological, anatomical, and embryo studies. In 1939 Sinnott,[5] in a study of growth and differentiation in living plant meristems, reported two quite different types of root-hair development among the members of the Gramineae which he examined. In one type, a cell which is to produce a root hair (the trichoblast) is definitely set apart as the apical, shorter, and more densely protoplasmic member of a pair of unequal daughter cells arising from the last division of one of the surface cells. When elongation has ceased, this cell produces a root hair, but root hairs are never formed from the longer sister cells. The cells in the surface layer are in longitudinal rows, and examination reveals that each row consists of short and long cells alternating. In the other type, however, all of the cells are essentially equal in size from the beginning and without any noticeable difference in the density of their protoplasmic contents, and are all apparently capable of producing root hairs. In a supplementary paper Sinnott and Bloch[6] supplied the additional information that in those grasses in which the root-hair initials arise by unequal divisions, the root hairs originate close to the apical end of the trichoblast and project forward at an angle of about 45° with the axis of the root. In those in which the divisions are equal, on the other hand, the root hair arises from about the middle of the cell and grows nearly straight out from the root at an angle of 90°. Thus there is a correlation between the type of cell division, the kind of cell from which the root hair originates, the point of emergence of the hair, and the angle at which this hair grows with reference to the root axis. For purposes of discussion we shall refer to the first type as type A, and to the second as type B (Fig. 1).

The grasses which Sinnott and Bloch used in their studies were species of the genera Poa, Phleum, Agrostis, Chloris, and Sporobolus. They reported that in the first three the root-hair development is of type A,[7] whereas in the last two it is of type B. In a later paper Bloch[8] reported that in *Phalaris arundinacea* the cell divisions are of type A, and while he did not specifically mention the angle of the root hair, his figure indicates that this characteristic would fit type A also. The traditional classification places all these six genera in the subfamily Festucoideae as members of four closely related tribes. Examination of the embryos of representatives

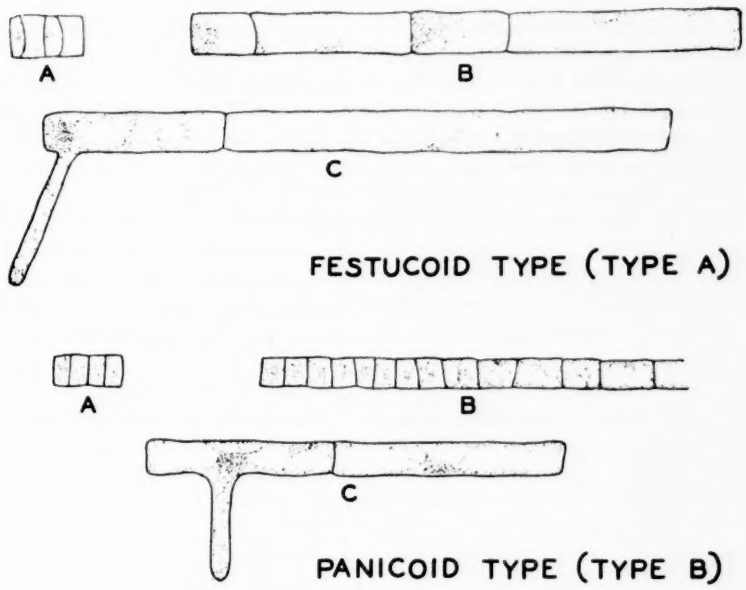

FIGURE 1

Camera lucida drawings showing development of root-hair initials and root hairs. The root apex is toward the left. Above, festucoid type (type A); below, panicoid type (type B). *A*, two pairs of cells in the meristematic region; *B*, the same cells during expansion; *C*, a root-hair cell and a hairless cell at maturity. (Figure from Sinnott and Bloch.)

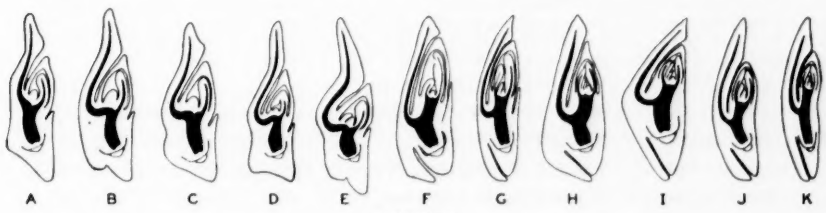

FIGURE 2

Median sagittal sections of embryos of several species of grasses. *A, Poa pratensis; B, Phleum pratense; C, Agrostis alba; D, Phalaris arundinacea; E, Beckmannia syzigachne; F, Sporobolus neglectus; G, Chloris verticillata; H, Panicum capillare; I, Digitaria sanguinalis; J, Miscanthus sinensis; K, Andropogon scoparius.* *A–E*, festucoid type. Note the lack of a cleft between the lower part of the scutellum and the coleorhiza and also that the point of divergence of the scutellum bundle is directly below the coleoptile. *F–K*, panicoid type. Note the prominent cleft between the lower part of the scutellum and the coleorhiza and also the distinct elongated region between the point of divergence of the scutellum bundle and the coleoptile. These drawings were made with the aid of a micro-projection drawing apparatus and are not all to the same scale.

of these genera, however, reveals two quite distinct types. Those of Poa, Phleum, Agrostis, and Phalaris are strikingly similar. They all lack a cleft between the lower part of the scutellum and the coleorhiza, and the point of divergence of the scutellum bundle is directly below the coleoptile. The embryos of Sporobolus and Chloris, on the other hand, are quite different from those mentioned above but are very similar to each other. These embryos have a prominent cleft between the lower part of the scutellum and the coleorhiza, and there is also a distinct elongated region between the point of divergence of the scutellum bundle and the coleoptile (Fig. 2). The first four genera also have a characteristic type of leaf anatomy and their chromosomes are large and the basic number is 7 (festucoid type). The two others have a different type of leaf anatomy and their chromosomes are small and the basic number is 9 or 10 (panicoid type). The structure of the embryo, the leaf anatomy, and the chromosome size and number thus show a close correlation and this appears to be evident also with respect to the type of root-hair development. Since it has been shown that the differences in anatomy, cytology, and embryo structure are subfamily differences, it seemed reasonable to suppose that the two types of root-hair development might also be of this same order. The data of Sinnott and Bloch suggest that type A of root-hair development might be characteristic of the Festucoideae and type B of the Panicoideae.

TABLE 1

	POA	PHLEUM	AGROSTIS	PHALARIS	BECKMANNIA	SPOROBOLUS	CHLORIS	PANICUM	DIGITARIA	MISCANTHUS	ANDROPOGON
ROOT											
Cell divisions: Type A or B	A	A	A	A	A	B	B?	B	B	B	B
Position of hair: apical (A) or median (B)	A	A	A	A	A	B	B?	B	B	B	B
Angle of hair (approximate)	45°	45°	60°	45°	45°	90°	80°	90°	90°	90°	90°
LEAF											
Epidermis	F	F	F	F	F	P	P	P	P	P	P
Anatomy	F	F	F	F	F	P	P	P	P	P	P
Embryo type	F	F	F	F	F	P	P	P	P	P	P
Chromosome basic number	7	7	7	7	7	9, 10	10	9	9	9?	5 (10)

NOTE: Data on root development compiled from Sinnott and Bloch, except for the genera Beckmannia, Panicum, Digitaria, Miscanthus, and Andropogon; data on leaf epidermis and anatomy from Prat; and chromosome numbers from Darlington and Janaki Ammal, Chromosome Atlas of Cultivated Plants. (F = festucoid type; P = panicoid type.)

Since none of the grasses mentioned above are placed in the Panicoideae under the traditional system, it seemed desirable to study the root-hair development in some undisputed members of this subfamily. Accordingly seeds of *Panicum capillare*, *Digitaria sanguinalis*, *Miscanthus sinensis*, and

Andropogon scoparius were germinated, following the techniques described by Sinnott, and the development of the root hairs studied. The first two species are members of the tribe Paniceae and the last two are members of the Andropogoneae. No one would question the fact that members of these two tribes are "true" Panicoideae. As was expected, the root-hair development was of type B. There were no significant differences in the sizes of the cells produced at the last cell division, and the root hairs arose from about the middle of the cells and projected out at an angle of essentially 90° from the root axis.

Root-hair development was also studied in the germinating seeds of *Beckmannia syzigachne*. This genus is of interest since it has been considered traditionally as a member of the Chlorideae although it differs from other members of that tribe in embryo structure, leaf anatomy, and chromosome size and number. Several authors have suggested that it should be removed from the Chlorideae, the members of which show panicoid characteristics, while those of Beckmannia are festucoid. As we had expected, it was found that the root-hair development in Beckmannia is of type A, while in the genera of true Chlorideae, as far as we have been able to determine, it is of type B. (Table 1 shows the correlation which exists between root-hair development and cytological, anatomical, and embryo characters in the genera studied.)

While in most of the species which have been examined the type of root-hair development is quite distinctive and is clearly either one or the other of the two types discussed, one discrepancy should be noted. This involves the genus Chloris which Sinnott and Bloch reported as being somewhat intermediate between the two types. At the last cell division, they stated, one of the cells is slightly smaller than the other, but: "this difference is by no means as great as in Phleum and Poa." They also reported that the root hairs occur at about a fourth of the way back from the apical end and tend to bend forward slightly. The root-hair development, therefore, while being more like type B, is somewhat irregular and not as clear-cut as in the other genera. It seems significant that Prat, although indicating that the anatomical characteristics of the Chlorideae are panicoid, states: "Toutefois, ils se distinguent par quelques différences de détail qui suffisent à déterminer un sous-type d'organisation que nous pourrons appeler *chloridoïde*."

The data of Sinnott and Bloch, supplemented by our own studies, indicate that the type of root-hair development within members of the Gramineae may have some important phylogenetic significance. These differences are easy to observe as one needs only to germinate the seeds and to examine the living roots under a compound microscope. In fact, the angle of the root hairs may be seen with the naked eye, or with but slight magnification, and this angle gives a clue to the type of division within the cells.

Studies of this nature may yield additional information which will aid in the proper placement of puzzling genera within the large and taxonomically difficult grass family.

Summary.—It is suggested that the two types of root-hair development noted by Sinnott and Bloch within the Gramineae may have important taxonomic significance. Studies with additional genera are reported, and it is revealed that there is a close correlation between the type of root-hair development, the structure of the embryo, leaf anatomy, and size and number of chromosomes. This evidence further emphasizes that anatomical and cytological characters appear to give more certain clues to the true affinities of genera in the Gramineae than do those gross morphological features traditionally used.

* This research was aided by a grant from the National Science Foundation.

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[2] Prat, H., *Ann. Sci. Nat. Botan., X.*, **18,** 165–258 (1936).

[3] Tieghem, P. van, *Ibid., VIII*, **3,** 259–309 (1897).

[4] Reeder, J. R., *Bull. Torrey Botan. Club*, **80,** 187–196 (1953).

[5] Sinnott, E. W., PROC. NATL. ACAD. SCI., **25,** 55–58 (1939).

[6] Sinnott, E. W., and Bloch, R., *Ibid.*, **25,** 248–252 (1939). See also *Am. J. Bot.*, **26,** 625–634 (1939).

[7] In his first paper Sinnott, while reporting that the divisions in the root tips of Agrostis were like those of Phleum and Poa (type A), did not mention what species he used and in his succeeding papers this genus is not mentioned. Since he gave no information concerning the position or angle of the root hair, we secured seeds of *Agrostis alba* and *A. tenuis* in order to check these points. The roots of these species showed distinct type A cell divisions, but in the region of elongation, especially near the root-hair zone, the difference in cell size is not as striking as in our other type A material. The root hairs, however, occur near the apical end of the cells and form an angle of about 60° with the axis of the root.

[8] Bloch, R., *Bull. Torrey Botan. Club*, **70,** 182–183 (1943).

*THE MOLECULAR WEIGHT OF SOME LONG CHAIN SALTS IN BENZENE BY THE CRYOSCOPIC METHOD**

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When salts are dissolved in benzene, whose dielectric constant at 25° is 2.28, they are ionized to the extent of one part per million or less at lower concentrations. Up to 1×10^{-4} N, the salts exist as ion-dipoles or ion-pairs. At a concentration of 1×10^{-3} N, all salts begin to form more complex structures, such as quadrupoles and even more highly associated molecular species.

The dipoles of salts which have two large unsymmetrical ions and whose dipole moments are not too large, such as tributyl- or triamylammonium picrate,[1, 2] exhibit but little association. For these salts an equilibrium exists between dipoles and quadrupoles which conforms to the law of mass action.[3] If one of the ions of the salt is small, as with tributylammonium iodide, associated molecules of a higher order than quadrupoles are formed, even at low concentration.[1]

The quaternary ammonium salts, such as tetrabutylammonium thiocyanate, which have large dipole moments, have association numbers greater than two, even at 1×10^{-3} *N*. For these salts, the association numbers increase with increasing concentration. According to Copenhafer,[4] for example, the association number of tetraisoamylammonium thiocyanate increases to a maximum value of 26 at a concentration of approximately 0.12 molal, after which it decreases to a value of 11.5 at 0.426 molal. Similar results have been obtained for several other salts.

Copenhafer found that the association number of octadecyltributylammonium formate increases from a value of 10, at 10^{-3} *N*, to 20, at 3×10^{-3} *N*. Young[5] found the association number of octadecyltributylammonium thiocyanate to increase to a value of 40 at 0.15 *M*, with no indication of a maximum.

It seems that the salts of long chain ions are more highly associated than are salts of symmetrical ions. Accordingly, we have determined the molecular weight of octadecyltri-*n*-amylammonium thiocyanate, octadecyltri-*n*-butylammonium bromate, and octadecyltri-*n*-amylammonium formate.

Measurements were carried out by the method developed by Vingee[6] with minor modifications as developed in subsequent investigations. The method consists essentially in measuring the temperature difference between the freezing point of a solution and of pure benzene by means of a sensitive thermal unit and determining the concentration of the solution by drawing off weighed samples and weighing the salt on evaporation of the solvent.

Two cells, of approximately 350 cc. capacity and provided with suitable covers, are placed in silvered Dewar tubes and the whole system is immersed in a thermostat whose temperature is controlled to slightly below that of the solution to be measured. The cells are provided with stirrers of the propeller type which are geared together so as to rotate at the same rate (about 350 r. p. m.).

The 36-junction thermel units are contained in platinum-tipped German silver tubes which extend into the body of the liquid. The thermel was sensitive to 0.00001°C.

About 225 cc. of pure benzene is introduced into each cell and about one-third is frozen out. The solid is reduced to a fine powder by means of the stirrers.

When temperature equilibrium has been established at the end of one-half hour or more, as indicated by the thermel, a known weight of solute is introduced into one of the cells and the temperature of the bath is adjusted. When equilibrium has again been attained, the e. m. f. of the thermel is measured. The stirrers are then stopped and the solid is allowed to settle to the bottom. Two samples of the solution are withdrawn into weighed quartz pipettes and the weight of each solution is determined. The solu-

TABLE 1

CRYOSCOPIC DATA FOR SOME SALTS IN BENZENE SOLUTION

MOLAL CONC.	$\Delta T°C.$	APPARENT MOL. WT.	ASSOC. NO.
A. *n*-Octadecyltri-*n*-amylammonium thiocyanate. Formula wt. 538.98			
0.00220[a]	0.00320	1885	3.50
0.00661[a]	0.00612	2962	5.50
0.08393[a]	0.02046	11250	20.87
0.09559	0.02269	11550	21.43
0.1122	0.03149	9769	18.12
0.2120	0.1111	5234	9.71
B. *n*-Octadecyltri-*n*-butylammonium bromate. Formula wt. 566.74			
0.00205	0.00617	958	1.69
0.00283	0.00855	955	1.68
0.00472	0.01393	976	1.72
0.01059	0.02555	1195	2.11
0.05299	0.06677	2289	4.04
0.1097	0.08564	3694	6.51
0.1464	0.09872	4275	7.58
0.2007	0.1371	4218	7.45
0.2703	0.1904	4094	7.22
0.2817	0.2070	3925	6.92
0.4891	0.3873	3641	6.42
C. *n*-Octadecyltri-*n*-amylammonium formate. Formula wt. 525.92			
0.00082	0.00105	2089	3.97
0.00141	0.00184	2041	3.90
0.00318	0.00350	2431	4.62
0.00600	0.00485	3310	6.29
0.01008	0.00660	4084	7.77

[a] Data of Young.[5]

tions are evaporated by means of a stream of air, the pipettes are exhausted to remove residual solvent and the pipettes are reweighed. With the long chain salts, it is difficult to remove the benzene completely by pumping. Accordingly, the sample is melted under vacuum and exhausted. After freezing under vacuum, it is again melted. This process is repeated until the melting point of the salt is within 1.0° of the melting point of the pure salt and the weight is within 0.1% of that of the preceding cycle. Using

known weights of octadecyltributylammonium bromate, it was found that the removal of solvent was complete at the end of three cycles.

Knowing the concentration of the solution and the temperature depression due to the solute, the molecular weight may be computed on the assumption that Raoult's law holds. While the weight concentration of solute may be rather high, the solutions are not very concentrated in a thermodynamic sense since the association of the solute is high and the total number of molecules in solution is not great.

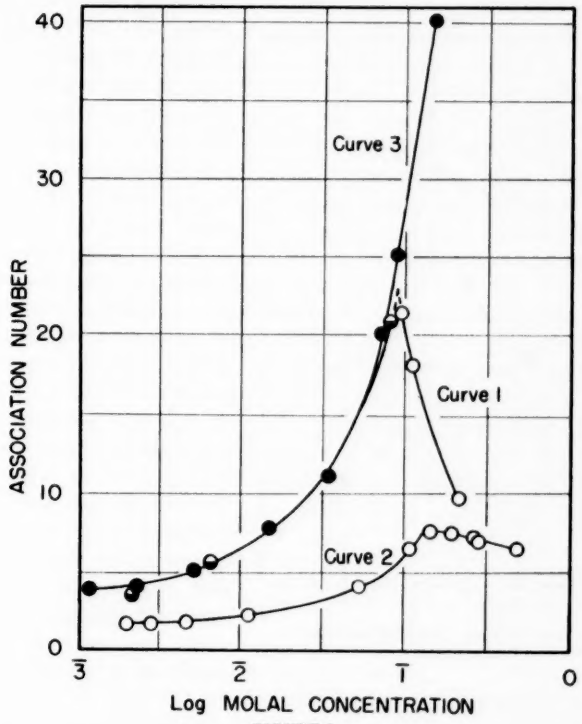

FIGURE 1

The results for octadecyltri-*n*-amylammonium thiocyanate are presented in table 1A; for octadecyltri-*n*-butylammonium bromate, in table 1B; and for octadecyltri-*n*-amylammonium formate in table 1C. In these tables, the concentration of the solutions, in moles per 1000 g. of benzene, is given in column 1, the freezing point depression, ΔT, in column 2, the apparent molecular weight in column 3, and the association number n, the number of formula weights per mole, in column 4. In computing the molec-

ular weights, the freezing point constant of benzene has been taken to be 5.085°.[5]

Association numbers for octadecyltriamylammonium thiocyanate are shown graphically on curve *1*, figure 1 (open circles). Those for octadecyltributylammonium bromate are shown on curve *2*. Young's[5] association numbers for octadecyltributylammonium thiocyanate are likewise shown on curve *3* (black circles) and his values for the amyl derivative are shown on curve *1* (half-shaded circles).

Up to a concentration of 0.08 molal, the curves for the amyl and butyl derivatives differ but little. However, at higher concentrations, the association numbers of octadecyltributylammonium thiocyanate increase continuously to a value of 40 at a concentration of 0.15 molal while that of the corresponding amyl salt passes through a maximum value of about 23 at a concentration of approximately 0.09 molal. Thereafter, its association number decreases to a value of 9.7 at a concentration of 0.21 molal. The two branches of the curve to either side of the maximum are convex toward the axis of log *m* and it is probable that they intersect at the maximum. The curve for the butyl derivative could not be carried to a maximum because of the limited solubility of this salt.

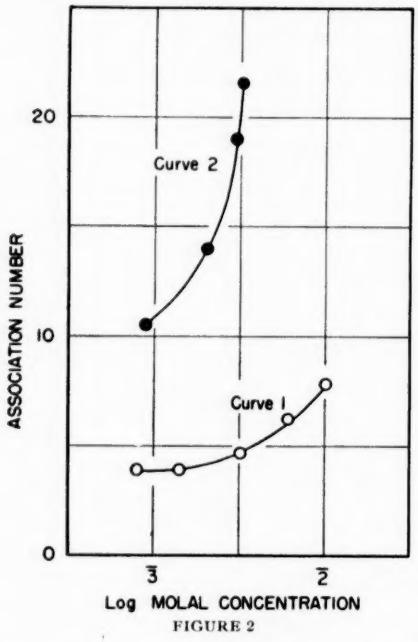

FIGURE 2

The association numbers for octadecyltributylammonium bromate are much lower than those for the corresponding thiocyanate. The curve for the bromate resembles that of octadecyltriamylammonium thiocyanate in that the curve consists of two branches which interact at a maximum of 7.55 at a concentration of 0.146 molal. The branch toward higher concentration is a linear function of log *m*, the branch toward lower concentration is convex toward the log *m* axis.

The curves for salts of ions having long chains differ markedly from those of salts of symmetrical ions, such as tetrabutylammonium thiocyanate.

The association number of the latter salt exhibits a well-rounded maximum of 32 at a concentration of 0.28 molal. It seems probable that the differences found in the behavior of these salts at high concentration are due to the presence or absence of the octadecyl group in the positive ion.

The association numbers of octadecyltriamylammonium formate are shown graphically in figure 2, curve *1;* Copenhafer's values for octadecyltributylammonium formate are shown as curve *2* in the same figure. Although the association of the amyl derivative is marked, it is much less than that of the corresponding butyl derivative.

It may be noted that with these salts of high molecular weight, any impurity that might be present would tend to lower the observed value of the molecular weight and, correspondingly, the association number.

Comparatively small changes in the substituent alkyl groups lead to marked changes in the degree of association. We see this illustrated in the case of the formates. The effect is likewise apparent in the thiocyanates of the octadecyltributyl- and octadecyltriamylammonium ions. The two salts are almost equally associated at lower concentrations but, at higher concentrations, the amyl derivative exhibits a sharp maximum at 0.09 molal while the association number of the butyl derivative continues to increase up to 0.15 molal, the limit of solubility.

The forces responsible for the association of salts in benzene are chiefly due to the charges on the ions, although van der Waals' forces may also be involved. The degree of association is greatly dependent on the size and shape of both ions. The interpretation of cryoscopic data in benzene in terms of association yields a coherent picture of what is going on in these solutions. While the detailed mechanism of the association process cannot be formulated in most cases, there can be little doubt but that association is chiefly responsible for the cryoscopic behavior of electrolyte solutions in benzene.

* This paper is based on a portion of a thesis submitted by Charles H. Keith, in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the graduate school of Brown University, July 1951.

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[4] Copenhafer, D. T., and Kraus, C. A., *Ibid.*, **73,** 1557 (1951).
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ACTION OF BORON TRIFLUORIDE ETHERATE ON β-CAROTENE

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Although it had been observed some time ago that boron trichloride[1] and trifluoride[2] form dark blue complexes with carotenes, nothing seems to be known about the products that result when such complexes are broken. We have investigated this conversion to some extent by a cleavage of such complexes with methanol and subsequent chromatographic resolution of the product. It was observed that the composition of the reaction mixture thus obtained from β-carotene is greatly dependent on the length of time that elapses between the formation and splitting of the complex.

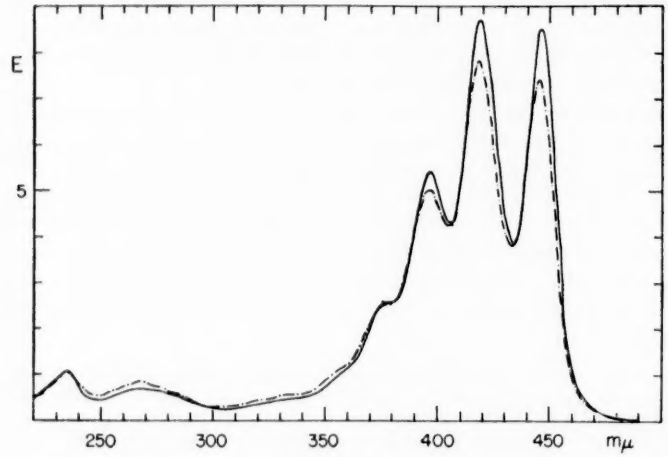

FIGURE 1

Extinction curve of Pigment I in hexane solution: ——, fresh solution; and – · – · –, after iodine catalysis, in light.

When the duration of the experiment was only one minute, the cleavage product of the complex was composed mainly of stereoisomeric β-carotenes; about 36% of the pigment remained in the all-*trans* configuration, while 44% assumed various *cis* forms. In fact such treatment is an efficient tool for the preparation of stereoisomeric carotenes. Only 1% of the initial substance was converted into pigments not belonging to the stereoisomeric

β-carotene set, and 19% remained unidentified, including (in part) colorless substances that showed intense fluorescence in ultra-violet light.

While these results remained essentially unchanged when the interaction of β-carotene and boron trifluoride etherate was extended to 5 minutes, an entirely different result was obtained after a full hour's standing of the complex at room temperature. Then approximately 40% of the starting material was present in form of pigments (estimated photometrically as "β-carotene"); however, β-carotenes were absent. The colorless portion was strongly fluorescent.

On the chromatographic column the pigment mixture was divided into four main zones and a number of minor fractions. The content of two

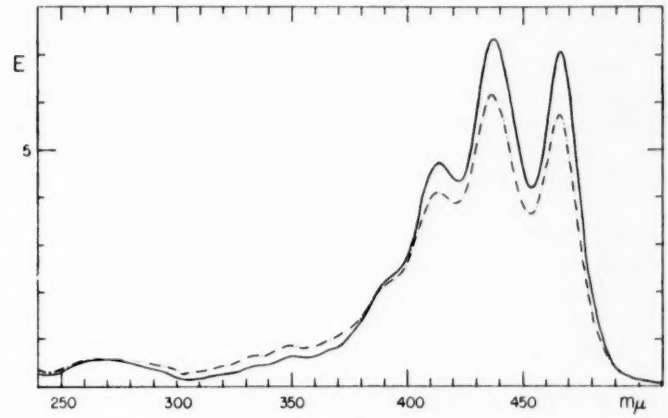

FIGURE 2

Extinction curve of Pigment II in hexane solution: ——, fresh solution; and – · – · –, after iodine catalysis, in light.

main zones could be crystallized, viz., Pigment I, m.-p. 214°, quadrangular plates; and Pigment II, m.-p. 159°, forming similar but much smaller units.

The extinction curves of these new polyenes demonstrate that a considerable shortening of the β-carotene chromophore has taken place. The position of the spectral maxima seems to indicate the presence of 8 or 9 conjugated double bonds instead of 11 (Figs. 1 and 2).

We are studying the interaction of some other carotenoids and boron trifluoride. It was observed recently that the sensitivity of individual carotenoids towards BF_3, varies within broad limits. Thus, as will be reported elsewhere in more detail, dehydro-β-carotene, $C_{40}H_{54}$, disappears when treated with the etherate for one minute.

Experimental.—About 20 mg. of β-carotene dissolved in 25–40 ml. of

hexane was shaken with 2 ml. of boron trifluoride etherate, $BF_3 + (C_2H_5)_2O$ (b.-p. 124–126°), at room temperature. The dark blue complex was decomposed by the addition of 100–200 ml. of 95% methanol, then transferred with water into hexane. The hypophase was discarded and the epiphase washed, dried, adsorbed on a suitable lime-celite column (2:1) and developed with hexane. The respective zones were eluted with acetone and transferred into hexane. The *cis* members of the stereoisomeric β-carotene set were identified as such by catalyzing with iodine and showing that the resulting all-*trans* pigment did not separate in the mixed chromatogram test from β-carotene *ex* carrots. Pertinent spectroscopic observations were also made. The hexane solutions of pigment zones I and II were completely evaporated in a dry nitrogen stream; the light orange colored powdery residues were dissolved in the minimum amount of benzene and crystallized at 50° in a centrifuge tube by cautious addition of methanol (50°). The samples were recrystallized in the same manner. The curves given in the figures were taken in the Beckman instrument. The maxima, as observed visually in the Zeiss Evaluating Grating Spectroscope (Loewe-Schumm) and compared with corresponding data for β-carotene, were located as follows: Pigment I: 448, 422 mμ (in hexane); Pigment II: 468, 441 mμ; β-Carotene: 484, 454 mμ.

Summary.—β-Carotene when treated with boron trifluoride etherate for one minute yields mainly some of its *cis* forms; however, upon longer treatment several new pigments with shorter chromophores appear, two of which have been crystallized.

* Contribution No. 1806.

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*MENDELIAN AND NON-MENDELIAN FACTORS AFFECTING THE CYTOCHROME SYSTEM IN NEUROSPORA CRASSA**

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It was reported recently that the characteristics of a slow-growing strain, called *poky*, are inherited maternally.[1] This observation has focused attention on strains which grow slowly on complete medium and which have long been known to appear very frequently in material examined in order to detect nutritional mutants of Neurospora. An indication of the frequency of their appearance can be obtained from some figures taken from

the data of Beadle and Tatum.[2] Among 8795 ascospores isolated from irradiated material during their study by one of the present authors (M. B. M.), 7049 germinated. Of these, 54 gave rise to strains having easily diagnosed requirements for components present in the complete medium used and, therefore, represented the class of nutritional mutants sought. A much greater number, 489, were distinguishable from wild type because of their slower growth on complete medium. Of these 159 did not grow sufficiently to allow their being tested. The remaining 330 were discarded, either because they grew too rapidly on minimal medium, or because they did not give a well-defined response to the growth-factor mixtures present in the complete medium. Similar figures have been reported by Lein, Mitchell, and Houlahan[3] in a description of a variation in the method of Beadle and Tatum.[2]

The advent of *poky* has not only aroused curiosity as to the nature of the abnormalities associated with the slow growth of such strains but has also suggested ways in which an investigation of them might be started, namely, by learning the mode of inheritance and by examining the cytochrome system for abnormalities such as those exhibited by *poky*.[4, 5] Among slow-growing strains recently isolated and studied, three have been found which show abnormalities with respect to cytochromes. The differences from normal are inherited maternally in one of these strains and as gene mutations in the other two. The four strains, *poky* and the three to be described here, are all different phenotypically. Ephrussi and his collaborators[6] have described, in yeast, a segregational mutant and a vegetative mutant both of which are characterized by the same disturbances of the cytochrome and succinic oxidase systems. A strain of yeast described by Ycas and Starr[7] has, in addition to these disturbances, other abnormalities which are inherited as if due to gene mutation.

Inheritance.—The three Neurospora strains were found among single ascospore isolates from a cross of wild type Abbott 12a protoperithecia to wild 1400-4A conidia, both untreated. The spores were plated on minimal medium and examined after about 15 hours of incubation at 25° C. when slow-growing ones were isolated. Strains from six isolates showed maternal inheritance of the abnormalities but since they have not been shown to be different, only one, designated mi-3, will be considered here. (The designation abbreviates "maternally inherited," thus *poky* is mi-1.) Strains showing Mendelian inheritance of the mutant trait included one arginine mutant and the two nuclear gene mutants described here, designated C115 and C117.

The mi-3 character descends to all progeny of crosses of mi-3 protoperithecia to normal conidia and to none of the progeny of reciprocal crosses, normal protoperithecia to mi-3 conidia. (In designating crosses, the protoperithecial parent will be given first.) Asci were isolated from mi-3 ×

wild 1400-4A and the strains from the four spore pairs of one ascus (number 2010) were crossed to wild types 2293-2a and 2292-2A. Spores were plated on minimal medium, heat treated, incubated at 25°C. for 12 to 20 hours and counted as follows:

	WILD	SLOW	
mi-3 − 2010-1 a × wild	2	962	
mi-3 − 2010-2 A × wild	0	473	
mi-3 − 2010-3 a × wild	..	..	(not fertile)
mi-3 − 2010-4 A × wild	0	1368	
Wild × mi-3 − 2010-1 a	1044	9	
Wild × mi-3 − 2010-2 A	1154	5	
Wild × mi-3 − 2010-3 a	1113	3	
Wild × mi-3 − 2010-4 A	1059	7	

Crosses of strains derived from an ascus (number 2543) from the cross mi-3 −2010 − 4A × wild 2293-2a gave similar results, which appear below.

	WILD	SLOW
mi-3 − 2543-1 a × wild	0	902
mi-3 − 2543-2 A × wild	0	1269
mi-3 − 2543-3 a × wild	0	1327
mi-3 × 2543-4 A × wild	0	2071
Wild × mi-3 − 2543-1 a	1067	8
Wild × mi-3 − 2543-2 A	842	7
Wild × mi-3 − 2543-3 a	1103	15
Wild × mi-3 − 2543-4 A	1174	13

Very few spores which had failed to germinate were observed. Occasional wild offspring from mi-3 × wild crosses are attributed, as in the case of *poky*,[1] to the formation of a few wild protoperithecia derived from the fertilizing conidia. Slow progeny from wild × mi-3 crosses are thought to be due to late germination or to factors which cause slow growth but are independent of mi-3 since those which were isolated and tested did not behave like mi-3. Crosses of mi-3 × mi-3 gave only slow progeny among 11,318 spores plated as above.

Because of the similarities between *poky* and mi-3 it appeared possible that one is a modified form of the other. In order to learn whether a segregating modifier is involved reciprocal crosses were made between *po*-1720-1a and mi-3-2010-1A. Strains derived from the four spore pairs of each of five asci from each cross were cultured at 25°C. in liquid minimal medium in 125-ml. flasks. The lowest, highest, and average dry weights in mg. for each set of 20 six-day cultures are given below.

	poky × mi-3	mi-3 × *poky*
Lowest	2	36
Highest	24	59
Average	13	49

Dry weights from *poky* × mi-3 progeny are typical of those from *poky* × wild progeny and dry weights from mi-3 × *poky* progeny are likewise typical of those given by progeny of mi-3 × wild. From these results it ap-

pears that if a modifier accounts for the difference in growth rates of *poky* and mi-3, the modifier is itself inherited maternally. Hence, it may be supposed that *poky* and mi-3 differ from each other, as well as from wild type, by a maternally inherited trait.

Mutants C115 and C117 were crossed, as conidial parents, to wild and to several other mutants. Counts of spores plated on minimal medium are given below.

	MUTANT	WILD	WILD, %
Wild × C115	596	590	49.7
47403 × C115	1529	76	4.7
51602 × C115	1807	573	24.1
C94 × C115	1245	247	16.6
C118 × C115	1382	458	24.9
70007 × C115	1109	403	26.6
Wild × C117	1050	1035	49.6
47403 × C117	1770	565	24.2
51602 × C117	2207	198	8.2
C118 × C117	1339	401	23.0
70007 × C117	1378	452	24.7

The other mutants involved in these crosses are as follows:

C94	histidine[8]-linkage group A
47403	succinic acid[9]-linkage group A
51602	riboflavin[10]-linkage group B
C118	slow-growing-linkage group not known
70007	"colonial"[11]-linkage group D

In 59 asci from crosses of C115 and in 127 from crosses of C117 there was 1:1 segregation of the mutant gene and its wild allele. These data indicate that the slow-growth character is due in each case to gene mutation; and that the C115 locus is in linkage group A and the C117 locus in linkage group B.

C115 and C117 have not been observed to function as protoperithecial parents. Both strains form protoperithecia but when these are fertilized they either stop developing after enlarging slightly and turning brown or they do not appear to change at all. Occasionally a few mature perithecia appeared belatedly but these could have arisen from protoperithecia formed by the fertilizing strain. Those which have been examined, from crosses to wild, showed 1:1 segregation of mutant and wild. All crosses attempted between C115 and C117 isolates were completely sterile. It seems probable, however, since the two mutants show linkage to different groups, that they are genetically different.

Preparation of Recombinants.—C115 in *poky* and mi-3 and C117 in mi-3 were obtained from asci from crosses to *poky* and mi-3 protoperithecia. The presence of *poky* or mi-3 in the recombinant strains seems unquestionable since from each ascus examined, strains from the two spore pairs not carrying the mutant genes were typically *poky* or mi-3. The presence of the

mutant genes was demonstrated by the observation of 1:1 segregation of mutant and wild in asci and among random spores from crosses of the recombinants to wild-type protoperithecia. Counts of random spores germinated on minimal plates are as follows:

	WILD	MUTANT	MUTANT, %
Wild × C115 in *poky*	835	857	50.6
Wild × C115 in mi-3	759	752	49.8
Wild × C117 in mi-3	861	902	51.2

Several mutant isolates from asci from each of these crosses were tested and proved to have the same characteristics as the mutant parent in the cross to *poky* or mi-3.

So far no isolate of C117 in *poky* has grown enough to permit its being thoroughly tested and out-crossed. Segregation of the mutant gene could be observed among spore pairs from 24 asci from *poky* × C117 isolated and allowed to germinate on plates of minimal medium. Two spore pairs from each ascus had produced, after about 20 hours, the amount of growth typical of *poky* and, when transferred to agar slants, continued to grow and formed typical *poky* cultures. The other two pairs in each ascus had, after 20 hours, produced hyphae not much longer than the ascospore and, when transferred to slants, these hyphae, in most cases, grew very little more and finally disintegrated. In a few cultures growth continued very slowly but after three weeks the agar surface in 3-inch test-tube slants was not covered with mycelium. Two such isolates have been kept alive by transferring to fresh slants bits of agar containing mycelium. Recently it has been found that their growth rate can be increased slightly by varying the complete medium used.

Growth Characteristics and Differences in Content of Cytochromes.—The following are typical dry weights of mycelium obtained from four-day cultures of the various strains at 25°C. in 125-ml. flasks containing 20 ml. of minimal medium with and without a supplement consisting of 40 mg. per flask of Difco yeast extract. A number of isolates of each strain (with the exception of C117 in *poky*) have been tested.

	DRY WEIGHTS, MG.	
STRAIN	MINIMAL	YEAST EXTRACT
Wild type	75	95
poky	11	14
mi-3	40	53
C115	30	2
C117	24	26
C115 in *poky*	4	<0.5
C117 in *poky*	<0.5 (6 days)	14 (6 days)
C115 in mi-3	6	<0.5
C117 in mi-3	23	24

In older cultures in flasks and on agar slants, a difference in the growth rates of C115 in *poky* and C115 in mi-3 becomes apparent, the latter strain being faster. The inhibition of C115 by yeast extract has been characteristic of all isolates examined but no inhibitory substance has been identified. Flask cultures of C117 in *poky* were inoculated with small bits of agar containing mycelium since the isolates have so far produced very few aerial hyphae and conidia.

The differences in intensity of absorption bands due to cytochromes are indicated in figure 1. The observations were made at room temperature with a hand spectroscope on moist pads of mycelium grown for four days at

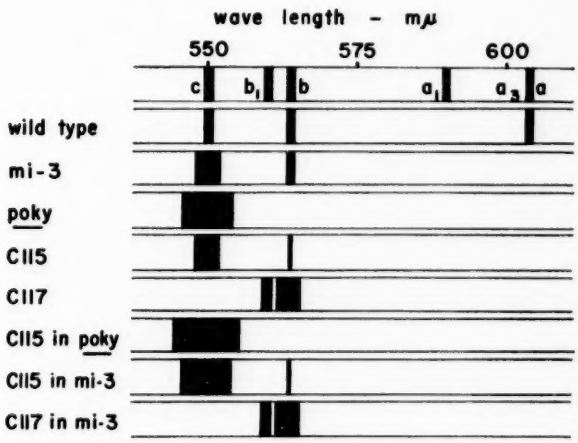

FIGURE 1

Diagrammatic representation of cytochrome absorption bands. Relative concentrations of cytochromes are indicated by the width of the bands, e.g., the ratio of cytochrome *c* concentration in wild type, mi-3 and *poky* is approximately 1:5:15.

25°C. on minimal medium in 125-ml. flasks. The pads were first treated with sodium hydrosulfite. Several cultures of the slower strains were used in order to obtain pads weighing about 30 mg. dry. It will be seen that *poky*, mi-3, and C115 are rather similar but the differences indicated, between cultures of this age, have been consistent. The *b* band is normal in mi-3, weaker than normal in C115, and very weak or not visible in *poky*. The *c* band is stronger in *poky*. The changes in *poky* which occur with increasing age have been described.[4] Briefly, older mycelium is more like that of wild type. This may be true to some extent, also, of mi-3 but not of C115 and C117. In C117 the *c* band has never been observed; the *b* band has been consistently stronger than normal; and the band which may cor-

respond to that of cytochrome b_1, not so far observed in any strain not carrying C117, has always been present. The absorption band of cytochrome *a* has not been observed in mycelium of this age from any of the strains. A band which may correspond to cytochrome a_1 is sometimes observed but this has not been consistent with any strain or even with any one isolate. It is usually seen in mycelium from mi-3, however.

With respect to content of cytochromes the characteristics of the recombinants are much as might be predicted from the properties of the individual strains. Cytochrome *c* is accumulated by *poky*, mi-3 and C115 and both C115 in *poky* and C115 in mi-3 show an increased accumulation, the greater increase in C115 in *poky* corresponding to the greater excess in *poky*. Since cytochrome *b* is normal in mi-3, weak in C115 and not detectable, by the method used, in *poky*, it is not surprising to find that this band is weak in C115 in mi-3 and not detectable in C115 in *poky*. That C117 in mi-3 does not differ from C117 is not surprising if, as it appears, C117 prevents the appearance of cytochrome *c* and induces an increase in cytochrome *b* in cytoplasm which, in the absence of the mutant gene, is normal with respect to *b*. On the basis of the characteristics of *poky* and C117 it might be predicted that only cytochrome b_1 could be detected in C117 in *poky*. In the few pads which have been examined the *a* and *c* bands could not be detected but a very weak and diffuse band in the region of *b* and b_1 could be seen. The band which may correspond to that of a_1 could be seen also, but it was weak. The behavior of the recombinants appears to emphasize the differences shown by *poky*, mi-3 and C115. Other differences have been demonstrated by comparisons of enzyme activities, to be reported elsewhere.

Mixed Cultures.—Mixed cultures have been studied in an attempt to learn whether these strains can interact to produce normal mold, but in no case has satisfactory evidence of such interaction been obtained. The mixture of *poky* and mi-3 was examined in particular since it appeared to offer more interesting possibilities. Different isolates, different conditions, and different means of testing the products (cultures established from hyphal tips and single conidia from the mixture and single ascospores from crosses in which the mixture was protoperithecial parent) were tried but no mold with a growth rate significantly higher than that of mi-3 was detected. It seems possible that the growth rate of a strain arising from a mixture of *poky* and mi-3 cytoplasms might be intermediate between that of *poky* and mi-3. Such a strain would be difficult to detect in the mixture

Summary.—Three strains of Neurospora, obtained from a cross of untreated standard wild types, show differences from wild type in growth rate and in cytochrome content which are similar to those exhibited by the *poky* strain, previously described. In one of these the character, designated mi-3, is, like *poky*, inherited maternally and the differences between *poky* and mi-3 appear to be inherited maternally. The other two characters,

designated C115 and C117, are inherited as gene differences. C115 and mi-3 both have an excess of cytochrome *c* but not as great as in *poky* and both, like *poky*, are deficient in cytochrome *a*. The content of cytochrome *b* is normal in mi-3 and low in C115 but not as low as in *poky*. Cytochromes *a* and *c* have not been detected in C117 but there is an excess of cytochrome *b*. The three strains grow more slowly than wild but faster than *poky*. Strains which carry the mutant genes along with the maternally inherited characters have also been examined. Their properties are much as would be predicted from the characteristics of the individual components.

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INDUCTION OF CHROMOSOME BREAKAGE AT MEIOSIS BY A MAGNESIUM DEFICIENCY IN TRADESCANTIA

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The mineral elements as constituents of the nucleus undoubtedly participate in important structural and metabolic functions of the nuclear components. Microincineration studies of Scott[1, 3] and Barigozzi[2] showed nuclear minerals, mostly calcium and magnesium, to be closely associated with the chromosomes. Allgén[4] found magnesium to be a constituent of nucleo-histone. Gulick[5] and, more recently, Milovidov[6] have reviewed the problem of inorganic elements within the nucleus. Poulson and Bowen[7] have used radioactive tracer methods in further explorations in this field.

A different method of approach is to test possible effects of alterations in mineral metabolism on the heritable components of the nucleus. Stubbe and Doring[8] found that sulfur, nitrogen, and phosphorus deficiencies increased the "spontaneous" mutation rate in the snapdragon. According to Demerec and Hanson,[9] a high level of manganous chloride considerably increased the mutation rate in *E. coli* (B/r).

The present report concerns the induction of chromosome stickiness by a magnesium deficiency. Because stickiness usually results in chromosome breakage, it is possible in this instance to specify the cytological mechanism responsible for alterations in the heritable material.

Materials and Methods.—Clonal material of *Tradescantia paludosa* (clone 5 of Sax) was grown by culture solution methods of Hoagland and Arnon.[10] Material for cytological examination was fixed in ethanol acetic acid mixtures (3:1) and stained by carmine or Feulgen smear techniques.

Culture Solutions.—In the first experiment, the low magnesium cultures were obtained by adding only limited amounts of magnesium sulfate to solution one.[10] The pH was adjusted weekly to 5.5 with sulfuric acid. The sulfate ion also served as the source of sulfur. For each treatment two sets of four plants each were grown in 7 liters of culture solution. Solutions were changed every two weeks. All material was grown at least three months.

In the second experiment the procedure for a magnesium deficiency was followed,[10] except iron was added as ferric potassium ethylenediamine tetra-acetate.[11] The pH was 5.8 at the start of cultures and before each change, which occurred every six weeks, the pH was no higher than 7.5. For each magnesium level, 10 plants were grown in 40 liters of solution. Most material was not fixed until 4 to 5 months after cultures were started.

Results: Observations on X-ray Control Material.—For the purpose of testing the sensitivity of the chromosomes to ionizing radiation, Tradescantia plants had been grown in culture solutions which were deficient or above normal for several different elements. The results on modification of the x-ray sensitivity will be published elsewhere. Non-irradiated microspores from a low magnesium culture (1.7 p.p.m.) possessed a relatively high percentage of micronuclei. In 422 apparently normal microspores there were 4.9% with one micronucleus and 0.48% with two. Along with the normal microspores there were 396 aborted cells (48.4%) in a total of 818. The micronuclei in the normal cells exhibited extreme variation in size which suggested chromosome breakage as the responsible mechanism. Examination of a limited number of meiotic stages gave evidence of chromosome stickiness. Mitoses in root tips and leaf bases showed neither chromosome aberrations nor sticky chromosomes.

Chromosome Stickiness and Fragmentation at Meiosis: First Meiotic Division.—A second series of low magnesium and magnesium deficient

plants was grown for more detailed analysis. Prophase nuclei were in a tight ball and difficult to spread. At pachytene and diplotene the chromosomes appeared to be somewhat vacuolated. At diplotene and diakinesis a few chromatids and chromosomes were broken. At metaphase I, chromosomes showed an extreme stickiness both within and between bivalents. In a few cases univalents were present but in a frequency of less than 1%. The conventional metaphase I figure, as in material from Hoagland's solution was rare in the magnesium deficient material. Bivalents from the controls were doughnut-shaped and separated normally, while magnesium deficient bivalents were in a tight mass and separated with difficulty. At anaphase I there were bridges and fragments, fragments alone, and chromatin bridges without fragments. The degree of disturbance was quite variable, within cells of the same anther and between anthers of the same bud. Immediately adjacent cells on the same slide had similar frequencies

TABLE 1

	TREATMENT	
	MAGNESIUM DEFICIENCY	CONTROL
No. cells	408	589
Chromosome fragments:		
1/cell	6.4	0.85
2/cell	1.2	0
3/cell	0.25	0
Chromatin bridges and fragments	1.96	0.34
Chromatin bridges (sticky bridges)	3.93	2.04
Attached fragments	0.74	0.17
Lagging chromosomes	0.74	0
Total chromosome fragments	12.2	1.36

NOTE: The frequencies of chromosome aberrations and nuclear disturbance were scored from anaphase II division figures. The table shows per cent of cells with the various aberrations.

of chromosome aberrations as if the effect were nonrandomly grouped.

Second Division of Meiosis.—In the second division the compact prophase and sticky metaphase chromosomes were present but the condition was much less severe than in the first meiotic division. A quantitative evaluation of the chromosome disturbances was possible at anaphase II. The data in table 1 show that the magnesium-deficient cells had a considerably higher frequency of chromosome aberrations than did the cells of the normal Hoagland's solution control. Chromosome fragments, the best estimate of breakage, were nine times more frequent in the magnesium deficient material. It was not always possible to distinguish between a chromatid or chromosome bridge and two unbroken chromosomes forming a chromatin bridge. For this reason all were classified as either chromatin bridges with accompanying fragments and chromatin bridges without fragments.

Tetrad and Early Microspore State.—The first sign of cellular lethality was observed in the very early microspore stage, and little if any cellular death had occurred before this time. Some samples from magnesium-deficient material exhibited 50 to 60% pollen abortion and in a few cases the cells were all aborted or disintegrated. The maintenance of normal cell function by the nucleus in individual microspores is undoubtedly autonomous after the tetrad stage. The cells which died after meiosis probably did so for at least two reasons: first, they did not possess all their essential chromatin, and secondly, the magnesium deficiency *per se* probably had some general adverse effect, such as that responsible for the small necrotic zones in root tips (see later section).

First Microspore Metaphase.—In the few hundred cells examined to date, the chromatid and chromosome breaks at the first mitotic division were less than 1%. Micronuclei were found in 4 to 6% of all cells examined. These mitotic figures and chromosomes were perfectly normal. No chromosome stickiness was present.

TABLE 2

	49 P.P.M. MAGNESIUM (CONTROL)	2 P.P.M. MAGNESIUM	1 P.P.M. MAGNESIUM	MAGNESIUM DEFICIENT
Total no. pollen	8229	5627	7026	6208
Total % of cells with micronuclei	1.03	1.07	1.98	2.00

	COMPARISON BETWEEN 49 AND 2 P.P.M	COMPARISON BETWEEN 1 P.P.M. AND DEFICIENT	COMPARISON OF 49 AND 2 P.P.M. BETWEEN 1 P.P.M. AND DEFICIENT
Chi-square	0.004	0.113	34.45
Probability at 1 D. F.	0.95	0.75	<0.001

Early Binucleate Pollen.—The counting of micronuclei in binucleate pollen is reasonably rapid and reliable for comparisons between different experimental treatments. However, an estimate of the percentage of chromosome fragmentation based on the per cent of micronuclei in the mature pollen grain would undoubtedly lead to a lower value than the real one because of genetic death of cells lacking essential chromatin. Table 2 shows the frequencies of micronuclei in four different levels of magnesium: magnesium deficient, two low-level cultures (1 and 2 p.p.m.), and normal Hoagland's solution control (49 p.p.m.). Eight or more buds from different inflorescences were scored per treatment. The frequencies in the deficient and 1 p.p.m. magnesium pollen were significantly higher ($P < 0.001$) than in the 2 p.p.m. magnesium and control cultures.

Samples from all treatments in both experiments were taken just previous to the onset of severe magnesium deficiency symptoms. The mean frequency of pollen abortion was 2.83% with a range of from 0.8 to 5.5% (12,520 pollen grains scored). With severe deficiency symptoms, the cell

samples which exhibited the highest percentage with micronuclei (5.5 to 6.5%) also had the highest frequency of aborted pollen. A combined count of 1029 microspores gave 50.2% aborted cells. The samples with fewer micronuclei (below 2%) taken from plants most severely effected by the magnesium deficiency had no more than 7.0% pollen abortion. These data then, indicate a positive correlation between the amount of pollen abortion and frequency of micronuclei in magnesium-deficient inflorescences. Such a correlation bears out the contention that an estimate of chromosome breakage produced at meiosis based on the frequency of micronuclei of magnesium-deficient pollen is too low.

Mature Pollen.—In nearly mature binucleate pollen which was severely affected by the magnesium deficiency, a number of bizarre cytological effects were observed. In both experiments a small fraction of the pollen, perhaps 1 to 2%, started precocious division of the generative nucleus. Stages from early prophase (where the chromosomes were visibly split), late prophase, and normal metaphase and anaphase figures were observed. Tri-nucleate pollen grains were the result of such precocious second divisions. In control pollen, either soil or culture solution grown, no such second mitotic division of the generative nucleus has been observed. In normal pollen, the second microspore division takes place in the pollen tube only after the pollen grain has germinated. Occasional mature generative nuclei in magnesium deficient pollen were broken into two or three sections. Other microspores exhibited various degrees of nuclear degeneration. Further study must be done to describe this particular aspect of the magnesium deficiency effect on the nucleus.

Somatic chromosomes from magnesium deficient root tips and leaf bases were examined. Little or no sign of nuclear or chromosome disturbances was found in leaf bases from the magnesium deficient plants. However, there were regions of dead cells in most deficient root tips.

Discussion.—The cytological effects produced by a magnesium deficiency are almost identical to those given by Beadle[12] in the description of the sticky gene in *Zea mays*. In both examples, first and second meiotic divisions are very sticky, and are followed by much degeneration of the microspores while the root tip mitoses appear normal cytologically. The fact that some disturbance of somatic divisions occurred in maize can be detected by the low vigor of the plants and by the presence of streaks of aberrant tissue; with the appropriate genetic factors chimeras could be produced. In addition, chromosome aberrations could be detected in some of the somatic metaphase complements. In the Tradescantia example, the mineral deficiency itself could account for all the observed effects on vigor, and for the presence of small necrotic zones in the root tips. Bands or sectors of aberrant tissue were not observed in the plants and no systematic study of mitotic complements has yet been made. In maize mutations

and chromosome aberrations have been found in the progeny of sticky plants,[13] and seed of the sticky Tradescantia material has been collected for a similar study.

A temperature shock was reported by Sax[14] to produce sticky chromosomes in Tradescantia. Other disturbances such as chromosome aberrations and precocious chromosome development of the generative nucleus were produced as well as asynapsis which in turn gave rise to diploid pollen. The temperature shock treatment caused a precocious division of the generative nucleus. La Cour[15] also found that temperature shock would produce further mitosis in dwarf pollen grains in Tradescantia.

The nuclear effects produced by the magnesium deficiency are in some respects similar to the temperature shock treatments. Just how these two treatments could be related is not clear. The possibility should be considered that a non-specific condition (i.e., starvation) resulting from the deficiency of any essential mineral could cause the meiotic chromosome disturbances produced by the magnesium deficiencies. Some evidence can be presented against such non-specific action of mineral treatments. The frequency of spontaneous chromosome aberrations of material grown in $^1/_{20}$ normal Hoagland's solution (starvation), in iron-deficient or in high-manganese solutions, was no higher than in controls (unpublished data).

As is well known, chromosome stickiness can be brought about by a number of additional treatments and conditions (i.e., light conditions and time of fixation, ionizing radiation, and certain chemicals). Himes[16] using photometric and cytochemical techniques (Feulgen and methyl green) found the sticky chromatin material in *Zea mays* and *Allium cepa* to be desoxyribose nucleic acid (DNA). Whether this DNA was polymerized or depolymerized is still open to question.[17] The sticky chromosome material of the magnesium deficient chromosomes may also be DNA, although this has not as yet been tested.

There is evidence to support the possibility that magnesium is directly concerned with the metabolism of DNA. Stephenson[18] reported that gram positive *Staphylococcus salivarius* when grown on minimal amounts of magnesium or on acid media became gram negative. It is generally conceded that nucleic acid (probably ribose nucleic acid) is the material stained in the gram stain reaction. Tamm and Chargaff[19] investigated the role of magnesium as a cofactor for desoxyribonuclease. Magnesium functioned here both in enzymatic and non-enzymatic break-down of DNA. The possibility that magnesium forms bonds between DNA molecules has been considered.[4] With magnesium present as a constituent of DNA and possibly responsible for the bonding of DNA molecules, the chromosomes would be expected to be more sensitive to breakage in absence of magnesium.

Magnesium, of course, functions as the coenzyme for a great variety of

enzymes in the cell. Of these, some are involved with phosphorylation (i.e., acid phosphatase reportedly inside the nucleus), photosynthesis, decarboxylation, glycolytic pathways (i.e., enolase), and others. Because of the cytological evidence presented, along with the pertinent findings concerning nucleic acid metabolism, the most reasonable hypothesis is that the sticky chromosomes and chromosome aberrations observed are due to the effect of the magnesium deficiency on some aspect of DNA metabolism. However, the possibility that other metabolic functions of magnesium could be responsible for the observed nuclear disturbances cannot be entirely discarded.

The interaction of magnesium with other mineral elements in the cell is extremely complex. For example, *E. coli* (B/r), treated with manganous chloride and then washed with magnesium chloride, had a reduced mutation frequency as compared to the manganous chloride treatment alone.[9] In other micro-organisms Abelson and Aldous[20] found that nickel, cobalt, zinc, and manganese interfered with the normal metabolism of magnesium. Steffensen (unpublished) showed that a high manganese nutrition reduced significantly the number of x-ray induced chromosomal aberrations in Tradescantia. With *E. coli* (B/r), the mutation frequency produced by manganous chloride plus ultra-violet treatment was lower than either one alone.[9] In both irradiation effects, it is not altogether certain whether manganese is involved with a primary effect or a secondary effect of interaction involving magnesium. The highly interrelated nature of mineral metabolism makes experimental interpretation difficult. A great deal of study with mineral elements and their effects on the nucleus must be done before any broad picture can be presented.

Summary.—In Tradescantia, a magnesium deficiency induced chromosome aberrations and stickiness at meiosis but not in mitosis. Chromosome fragments at anaphase II were nine times more frequent in deficient plants than in controls. Chromosome aberrations appearing at meiosis seemed to owe their origin to the stickness.

The frequencies of micronuclei in pollen from plants grown on magnesium deficiencies were increased significantly above controls.

Possible disturbances of DNA metabolism induced by a magnesium deficiency have been discussed.

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*UNITY OF THE VEGETATIVE POOL IN PHAGE-INFECTED BACTERIA**

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A considerable body of genetic[1–12] and chemical[13–18] facts has led to the following interpretation of the growth-cycle of T2 phage. Upon infecting a bacterial cell a phage particle is transformed into a noninfective intracellular form called *vegetative* phage. This vegetative form can multiply and, if a bacterium has been mixedly infected with two or more related phages which differ in their genetic composition, can also undergo genetic recombination. During the first few minutes after infection, called the *eclipse period*, only vegetative particles are present within the bacterium. Afterward infective (or *mature*) particles begin to form and accumulate at a constant linear rate until the bacterium lyses, at which time several hundred mature phage progeny are liberated. The vegetative particles are pictured as forming an intracellular *pool* from which units are removed at random and transformed irreversibly into mature phage particles, which then remain inert with respect to the multiplication and recombination processes of the vegetative particles. Thus the three critical processes which

are considered to be operating during phage growth are multiplication, recombination, and maturation of vegetative particles.

Recently it was found that it is possible to reinfect a cell as late as eight minutes after the primary infection if a very high dose of reinfecting phage (around 1500 particles per cell) is used.[19] By using T2 phage labeled with different genetic markers for the two infections, it was shown that the reinfecting phage type was represented in the progeny, and that all the recombinant types were also present with the frequencies to be expected for mixed infections with unequal amounts of parental phage. This discovery enables us to ask an additional question about the properties of the intracellular pool of vegetative particles, namely, whether or not the late-infecting phage will enter immediately into the vegatative pool which has already been established by the first phage. We will present evidence in this paper to show that the phages from both the early and the late infection multiply and recombine with equal facility within the same vegetative pool, and that the genetic markers introduced by the late infection are already represented in the first progeny which appear at the end of the normal eclipse period. Since the second infection can occur as late as eight minutes after the first, this means that the eclipse period for the second phage has been reduced to less than four minutes in contrast to the normal 12 minutes.

Materials and Methods.—In these experiments the following strains of bacteriophage and bacteria were used: T2 strain H phage, and its host-range mutant T2h and plaque-type mutant T2r; *E. coli* strain H bacteria (sensitive to all strains of T2), and the mutant *E. coli* B/2 (sensitive only to the h mutants of T2). These strains have all been described previously.[19, 20]

The nutrient broth and buffer media employed, and the layering technique used for obtaining plaque titers of the phage, have also been described previously.[19, 20]

Experimental Procedure and Results. (1) Single infection at time 0; multiple reinfection at 6 minutes or 8 minutes; premature lysis starting a 11 minutes.—*E. coli* strain H bacteria, grown in aerated nutrient broth at 37°C. up to a concentration of 10^8/cc., were centrifuged, washed once and resuspended in buffer, and then aerated at 37°C. for 20 minutes. Sufficient T2rh phage was added to give a ratio of one particle per bacterium, and 5 minutes was allowed for adsorption of the phage. Under these conditions there is no phage growth. The infected bacteria were then centrifuged and resuspended in nutrient broth at 37°C. to initiate phage growth (time 0 in the experiment). This suspension of infected bacteria was divided into three portions. The first provided a control, which was not reinfected; to the other two portions 1500 $T2r^+h^+$ particles per bacterium were added at 6 minutes and 8 minutes respectively. Starting at 10 minutes, aliquots were

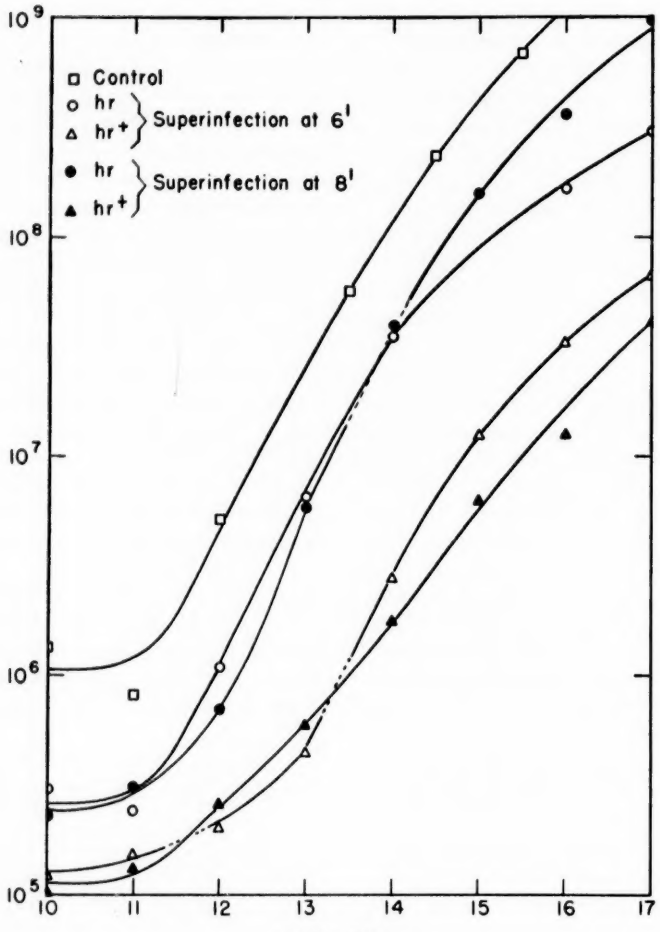

FIGURE 1

Rates of formation of the first parental type and one recombinant type progeny phage after reinfection (superinfection). The control was not reinfected. First infection with T2rh; reinfection with $T2^{+}h^{+}$. Ordinate (logarithmic scale): the numbers of plaque forming phage particles per cc. Abscissa: the times at which the infected bacteria were artifically lysed.

removed from each tube and diluted by a factor of 10,000 into a mixture of nutrient broth and 0.01 *M* NaCN. The cyanide stopped phage growth and lysed the bacteria, thus releasing the intracellular phage which was present at the moment of addition to the cyanide. After the cyanide dilution tubes were equilibrated for two hours at 37°C., samples were plated for plaques on *E. coli* B/2 bacteria. Of the four possible genetic types of

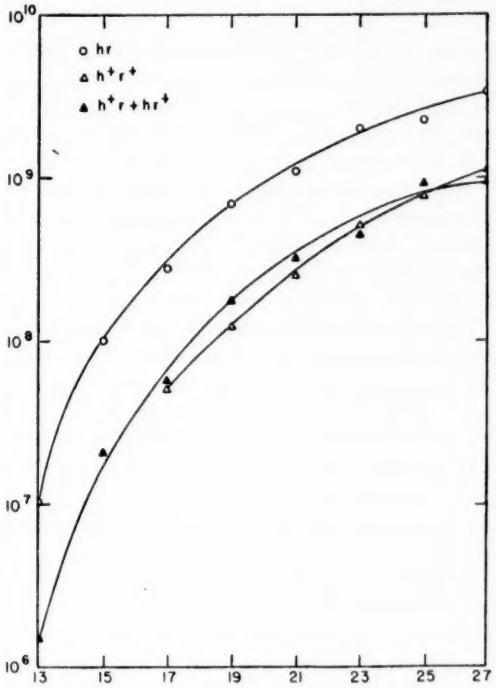

FIGURE 2

Rates of formation of the two parental types (hr and h^+r^+) and two recombinant types (h^+r and hr^+) of phage progeny after reinfection. Same units for the ordinate and abscissa as in figure 1.

phage in the lysates (parental rh and r^+h^+, and recombinant rh^+ and r^+h) only the rh and r^+h types will form plaques on B/2, and these can be readily distinguished from each other by their plaque morphology. The advantage of plating on B/2 lies in the elimination of the high background of plaques from unadsorbed r^+h^+ reinfecting phage; but there is the concomi-

tant disadvantage that only half of the four progeny types can be detected. The results of this kind of experiment are shown in figure 1, from which two conclusions may be drawn:

(*a*) Mature phage progeny first appear in the reinfected bacteria at the normal time (12 minutes) and thereafter accumulate at the normal rate.

(*b*) The r^+ genotype derived from the reinfecting phage is already present at 12 minutes (as r^+h recombinants) and also accumulates at the normal rate.

In order that all the genetic types formed might be detected, the above procedure was modified by adding anti-T2 serum to the infected cultures at 10 minutes (to eliminate the unadsorbed reinfecting phage) and then plating for plaques from the broth-cyanide lysate tubes on mixed-indicator bacteria (*E. coli* strain H and mutant strain B/2).[20] On mixed-indicator all the gentic types can be distinguished readily by the type of plaque formed. As a control, the lysates were also plated on B/2 bacteria alone, and since the same number of particles carrying the h genotype was found by both plating methods it is evident that there is no complication from phenotypic mixing (i.e., particles with h genotype but h^+ phenotype in this case.)[21] The results obtained with this procedure are shown in figure 2 for a case in which the bacteria were reinfected at 6 minutes. The rates of appearance of the two parental types and the two recombinant types have all been plotted. Since the observed number of recombinants of the complementary types r^+h and rh^+ was the same throughout the latent period, the values for the recombinants were pooled and only their totals plotted in figure 2. The h^+ progeny were not titrated until the 17-minute point in order to give the antiphage serum sufficient time to eliminate the unadsorbed reinfecting r^+h^+ phage. It should be noted in figure 2 that the total yield of recombinants during the latent period was generally below the yield of minority parental progeny (r^+h^+), contrary to the result obtained when bacteria are simultaneously infected with both parental types. This result is not unexpected, however, in view of the large variability under these conditions in the relative amounts of the two parental types infecting individual bacteria; in some bacteria the reinfecting phage is present as the majority instead of the minority parent.[19] We conclude that these data are entirely consistent with the idea of a single, homogeneous vegetative pool in which the first and reinfecting phages participate with equal facility; the reinfecting phage acts in most bacteria as the minority parent.

(*2*) *Reinfection at 6 minutes with ultra-violet-irradiated phage.*—Luria and Dulbecco[22, 23] discovered that if two or more phage particles which have been inactivated by ultra-violet (UV) irradiation infect a single bacterium, they can then cooperate (in some manner as yet unexplained) to produce viable progeny. This phenomenon is called *multiplicity reactivation*. We were interested in determining whether UV-inactivated phage,

upon reinfecting a cell 6 minutes after a first infection with viable phage, would be able to enter immediately into the vegetative pool (as is the case

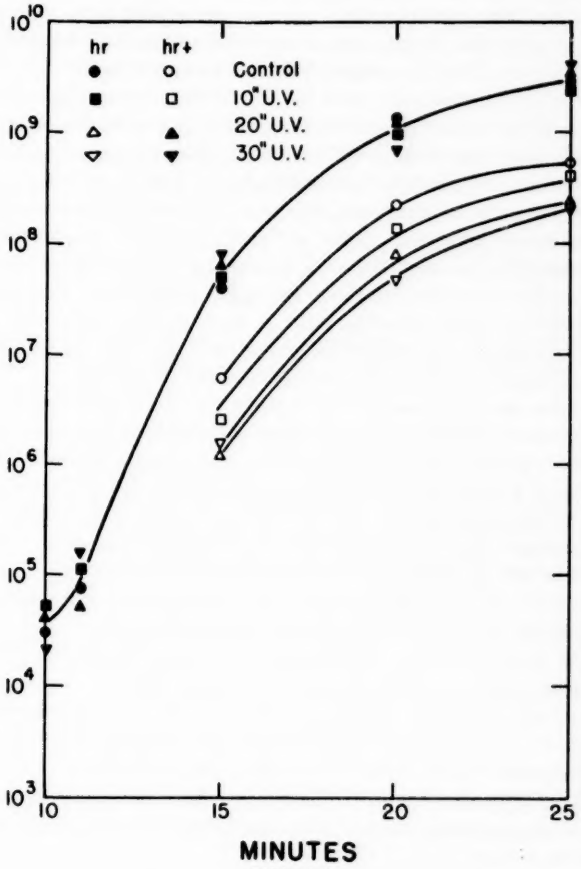

FIGURE 3

Rates of formation of the first parental type (hr) and one recombinant type (hr^+) of phage progeny after reinfection with ultraviolet inactivated r^+h^+ phage. The control was reinfected with viable phage. Same units for the ordinate and abscissa as in figure 1.

with viable reinfecting phage—see figures 1 and 2), or whether the need for multiplicity reactivation would cause some delay. For this experiment the same procedure was employed as in part 1. The experiment was

divided into four parts, with one control tube reinfected with viable phage, and three other tubes reinfected with inactivated phage which had been exposed to UV for 10, 20, and 30 seconds respectively. The phage was irradiated in buffer medium with a G. E. germicidal lamp delivering 10 ergs/mm.^{2}/sec.; the percentages of surviving phage after irradiation were 2%, 0.01%, and 0.0001% respectively. The first infection was with T2 rh phage and the reinfection with T2 r^+h^+ (3000 reinfecting particles per cell). In figure 3 the yields of the parental rh and recombinant r^+h types during the latent period have been plotted. As a control, the complementary recombinant type rh^+ was also titrated by plating on mixed-indicator cells, and the amount found was the same as for the r^+h recombinant. It is evident from the results shown in figure 3 that the genetic markers of both irradiated and nonirradiated reinfecting phage appear in mature progeny at the same time and increase at the same rate. The only effect of irradiation is to decrease the yield of irradiated markers. In all other respects UV-inactivated reinfecting phage can enter into the existing vegetative pool with the same facility as viable reinfecting phage.

Discussion and Conclusions.—In this work we have determined the rates of formation of mature T2 phage when a bacterium is subjected to two successive infections, with an interval of 6 or 8 minutes between infections. Since phages labeled with different genetic markers at two loci were used (T2 rh for the first infection, T2 r^+h^+ for the second) it was possible to follow individually the formation of phage progeny derived from both infections. The data presented reveal the following facts:

1. Reinfecting a bacterium after 6 or 8 minutes does not interfere with the normal growth processes of the first infecting phage.

2. The genetic markers introduced by the reinfecting phage appear in the progeny (both as parental and as complementary recombinant types) at the end of the normal eclipse period of 12 minutes, and thereafter accumulate at the normal rate. This is also true when the reinfecting phage has been inactivated by ultra-violet light. Since reinfection can occur as late as 8 minutes after the first infection, we have the phenomenon of new progeny being formed less than 4 minutes after the parental phage has infected a bacterium.

3. The complementary recombinant types (r^+h and rh^+) are produced in equal numbers throughout the period when mature progeny appear. Also the ratio of recombinant to parental-type progeny during this period is the value to be expected for mixed infections in which both parents can multiply and recombine on an equal basis. Under our conditions of reinfection the second phage behaves as the minority parent in most of the bacteria.

These results are readily interpreted in terms of an intracellular pool of vegetative phage particles (see the introduction). We picture the first in-

fecting phage as establishing the pool. The reinfecting phage then finds the pool available and enters immediately. Since the pool has probably been partly filled by the multiplication of the first phage before reinfection and only a few of the reinfecting particles are able to enter the cell, the second phage is in the minority. But once it has entered the pool the second phage can participate in the multiplication and recombination processes of the vegetative particles on an equal basis with the first phage. Consequently the events that follow reinfection are the same as if both infections had occurred simultaneously with unequal multiplicities of the two parental types; the eclipse period and the rates of formation of parental and recombinant progeny all have the normal values. Thus we conclude that even under extreme conditions of reinfection (either with viable or with ultra-violet-inactivated reinfecting phage) only a single uniform vegetative pool is established in a bacterium.

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INDUCTION OF MUTATIONS IN A BACTERIAL VIRUS*

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Introduction.—In the course of experiments designed for other purposes a paradoxical observation was made: phage λ, inactivated by UV irradiation, when adsorbed onto sensitive bacteria was reactivated when a further dose of UV was given to the phage-bacterium complexes. Among the reactivated phages a fairly large proportion were mutants. A description of these findings and a discussion of their implications will be found below.

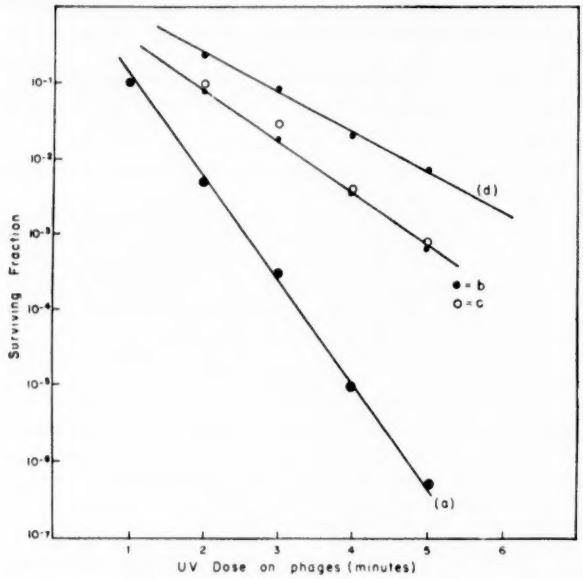

FIGURE 1

Semilogarithmic plots of the UV surviving fraction of irradiated phage λ plated (*a*) on non-irradiated bacteria, (*b*) on irradiated bacteria (60 sec., 10^{-1} survival).

Material and Methods.—Bacteria: *E. coli* strain K12 (λ), lysogenic for the temperate phage λ, and different derivatives called K12S, having lost the lysogenic character and having become thus sensitive to λ; *E. coli* strain C (Bertani and Weigle[1]). Phages: The temperate phages liberated by K12 (λ) and mutants of these phages, differing in the morphology of their plaques or in virulence.

The general methods used were those described by Adams.[2]

Ultra-violet irradiation of suspensions in buffer of bacteria or phages was done at a distance of 37 cm. from a 15-watt Sterilamp. At that distance, 8 sec. of irradiation leave a surviving fraction of 10^{-2} of the phage T2.

For visible light illumination, the plates were exposed at room temperature to the light of two parallel fluorescent lamps of 40 watts each at a distance of 30 cm. In a few experiments a General Electric H5 lamp of 250 watts was used (Dulbecco[3]).

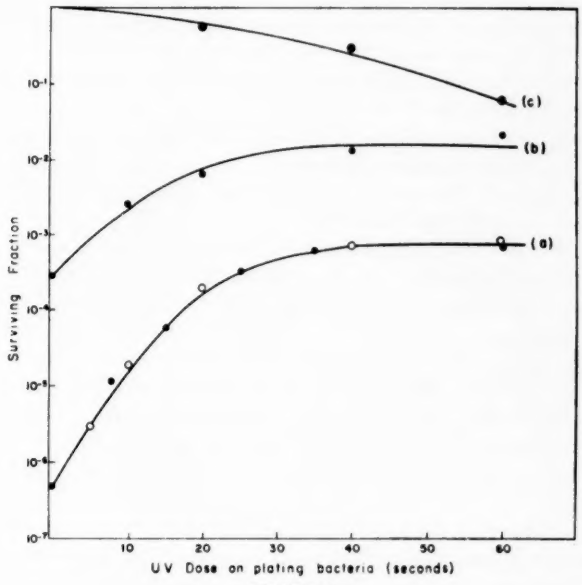

FIGURE 2

Semilogarithmic plots of the surviving fraction of λ phages having received a dose of (*a*) 5 min. UV irradiation (2 experiments), (*b*) 3 min. UV irradiation, as a function of the dose of UV given to the plating bacteria. The upper curve (*c*) shows the surviving fraction (as colony formers) of the irradiated bacteria.

Reactivation by UV'd Bacteria of Inactivated Phages.—Phage λ irradiated with UV and plated on *E. coli* strain K12S has a survival curve approximating a three-hit curve (Fig. 1, curve *a*). If the UV'd phages are plated on UV'd bacteria (dose of 60 sec., 10^{-1} survival as colony formers) the survival curve is still approximately a three-hit curve but the surviving fraction is much larger (Fig. 1, curve *b*).

The reactivation due to the UV'd bacteria depends on the dose of UV

received by the bacteria (Fig. 2). At low doses the reactivation increases with the dose. Saturation is reached at doses larger than 50 sec.; half of the reactivation is accomplished at a dose of 25 sec.

Reactivation can be obtained by irradiation of the bacteria either before adsorption of the inactivated phages to the bacteria or afterward (in the latter case the supplementary dose of UV given to the phages must be taken into account). If the complexes (UV'd phage-normal bacterium) are kept at room temperature in nutrient agar before irradiation, their reactivable half-life time is approximately 30 minutes.

If the irradiation of the bacteria has taken place before adsorption of the phages, the bacteria retain their reactivating ability for many days when kept in buffer at 4°C. The irradiated phages also retain for days their ability to be reactivated.

For non-irradiated phages the plating efficiency on irradiated bacteria is the same as that on normal bacteria.

The progeny of the reactivated phages has the same efficiency of plating on normal as on irradiated bacteria.

The UV-inactivated phages do not kill the bacteria. Even the reactivable particles do not kill the bacteria on which they are adsorbed.

The λ phages do not show multiplicity reactivation with the exception of the virulent mutant (Jacob[4]).

Reactivation (and the production of mutants to be described below) can be obtained not only by exposure of the bacteria to UV but also by their exposure to x-rays or to Dichlorene[5] (nitrogen mustard). These last two agents are known[6] to induce lysis in lysogenic bacteria as effectively as UV. Treatment of the bacteria with H_2O_2 does not produce reactivation (or mutations).

The treatment of the irradiated phages or of the non-irradiated bacteria or of both with the supernatant of an irradiated bacterial suspension does not produce reactivation. Neither does adsorption of UV'd λ on non-irradiated bacteria followed by plating on UV'd bacteria. The adsorption of the inactivated phage to the UV'd bacteria is thus necessary for reactivation to occur.

All the different mutants of λ have the same UV sensitivity. They are all reactivable by UV'd bacteria but the maximum amount of reactivation obtainable is slightly different for each of them.

All the mutant strains of the sensitive bacteria tested, differing in their nutritional requirements or having the F^+ or F^- character (see Hayes;[7] Cavalli, Lederberg, and Lederberg[8]) give reactivation (and mutations). *E. coli* strain C also produces both.

The reactivation by irradiated bacteria does not take place for UV inactivated T2, T3, or T5 (only T's tested) if plated either on UV'd strain B or UV'd strain K12S.

Appearance of Mutants Among the Reactivated Phages.—Mutants, producing plaques of strikingly different morphologies, are found among the reactivated phages. The parent type forms fairly turbid plaques because of the production of lysogenic bacteria which are resistant to the phage (Fig. 3). Six different types of mutants can easily be distinguished. Some give clear plaques, others give plaques which are more turbid than those of the parent type. Some of the mutants are unstable, giving rise, on subculture, to other mutants. With the exception of a few per cent, each plaque formed by a reactivated λ, showing a morphology different from that of the parent, contains only one type of phage.

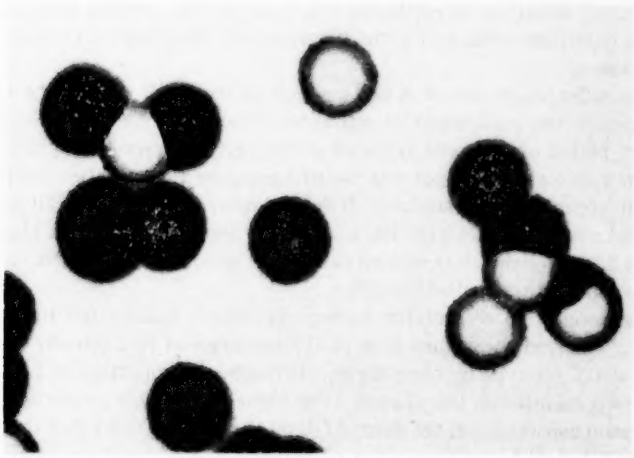

FIGURE 3

Plaques of the parent λ phage and of two different clear plaque-forming mutants, on 60-sec. irradiated bacteria. The parent produces turbid plaques; one of the clear mutant shows no growth in the center of the plaque while the other one does.

The mutants plate on normal and on irradiated bacteria with the same efficiency. They all have the same UV sensitivity and the same latent period as the parent. Their burst sizes, however, are different. They vary greatly from one another, and from the parent, in the efficiency with which they produce lysogenization of the bacteria they infect (this may be the main cause of the differences in the morphologies of their plaques). The mutants seem to be inactivated by heat at different rates.

When the mutants are inactivated with UV and reactivated by UV'd bacteria they, in turn, usually give rise to other mutants. Each mutant seems to have a characteristic mutation pattern. No spontaneous or in-

duced reversion of a mutant to the parent type has been observed. No host range mutant has been found. The virulent mutant although reactivable does not show any plaque morphology mutants nor does it give rise to host range mutants.

Some of the mutants appear spontaneously in the cultures of the parent phage with frequencies which are at most of the order of 0.05%. Mutants are also present in the lysate of lysogenic cultures induced with very high doses of UV.

No mutants are found (1) among the survivors of UV'd λ plated on normal bacteria, (2) when non-irradiated λ is plated on UV'd bacteria, (3) when UV'd λ is plated on non-irradiated bacteria and is photoreactivated, (4) when λC is plated on UV'd K12S (see Bertani and Weigle[1]). Thus, to obtain mutants, UV treatment of both the phages and the bacteria is necessary.

The different strains of K12S (as well as strain C) seem to be equally efficient in the production of mutants. Since the non-irradiated parent phage, plated on different indicator strains, gives plaques of slightly different morphologies, it is not practical to compare the mutation pattern on the different sensitive strains. It seems, however, that they all give the mutant whose plaques have the morphology seen in the clearest plaques of figure 3. Since the clear mutants are easily seen, only these were scored in the experiments described hereafter.

The proportion of mutants among the phages reactivated by bacteria having received a constant dose of UV increases at first linearly with the dose of UV received by the phages. It reaches a proportion of 2.5% for a dose of 5 minutes on the phages. For higher doses, this proportion seems to remain constant. If the dose of UV on the bacteria (and thus the reactivation) is varied, the proportion of mutants also varies linearly with the bacterial dose, reaching the maximum value of 2.5% for maximum reactivation (60 sec. of UV on the bacteria).

Photorestoration.—(*a*) Photorestoration of the UV'd bacteria—UV'd phages, plated on UV'd bacteria which have been illuminated with visible light, are not reactivated and show no mutants. The dose of light necessary to produce this effect is large and increases with the dose of UV received by the bacteria (one hour exposure to a G. E. H5 lamp is necessary to restore 15 sec. UV'd bacteria).

(*b*) Photorestoration of the complexes (UV'd phage-non-irradiated bacterium).—The dose of visible light necessary for maximum restoration of the (λ-strain K12S) complexes is approximately the same as that necessary for the restoration of (T2-strain B) complexes. The maximum amount of photorestoration is of the same order of magnitude as that obtained by plating on UV'd bacteria. When complexes are formed with different bacterial strains, the amount of photorestoration of one strain

does not differ by a factor larger than 10 from that of another strain (for the same dose of visible light).

For a constant, large dose of visible light the survival curve of the complexes is similar to a three-hit curve (Fig. 4). Among the reactivated phages no mutants are found.

(*c*) Photorestoration of the complexes (UV'd phage–UV'd bacterium).

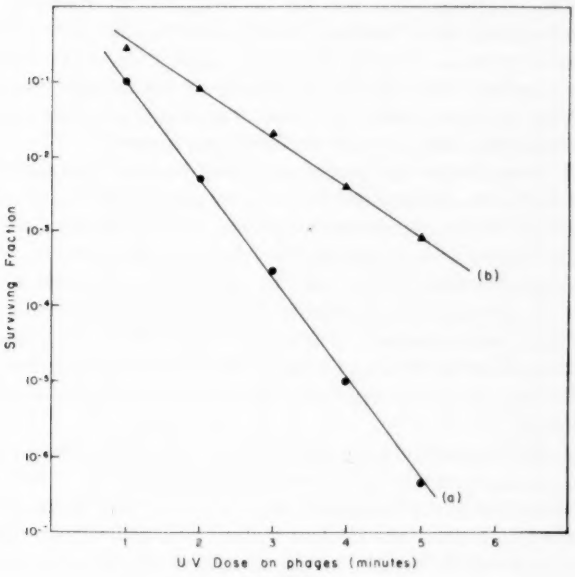

FIGURE 4

Photoreactivation of the complexes (UV'd phage-bacterium). Semilogarithmic plot of the surviving fraction of UV'd λ plated (*a*) on non-irradiated bacteria in the dark, (*b*) on non-irradiated bacteria and then illuminated for 3 hours at room temperature under the fluorescent lamps, (*c*) on 60-sec. UV irradiated bacteria in the dark, (*d*) on 60-sec. UV irradiated bacteria and then illuminated as in (*b*). The coincidence of curves (*b*) and (*c*) is fortuitous for they are separated when other bacterial strains are used for plating.

—Illumination with visible light of these complexes produces restoration and the survival curve is again a three-hit curve (Fig. 4). The maximum reactivation obtainable following both UV treatment of the bacteria and photorestoration seems to be the same for all the bacterial strains. Among the photorestored complexes the same type of mutants are found as before photorestoration but their proportion has decreased. The fact that, in

these conditions, some of the UV'd bacteria are restored and thus rendered unable to reactivate the phages, may be responsible for this decrease.

Discussion and Conclusions.—When temperate phages infect a sensitive bacterium they may provoke either a lytic or a lysogenic response. In the case of the lytic response the phage goes into the vegetative state, multiplies rapidly and the cell lyses after a definite latent period liberating newly formed particles. In the case of the lysogenic response the phage is reduced to the prophage state and the bacterial cell, by repeated division, gives rise to a clone whose bacteria all carry the prophage, that is the ability to lyse with liberation of phages upon the application of the right sort of inducing stimulus. This induction of lysogenic bacteria to lyse is caused in K12 (λ) by treatment with UV (15 sec. of irradiation produce induction in 99.9% of the cells), with x-rays or with nitrogen mustard.

After UV irradiation the phages are called inactive because they have lost their ability to multiply and thus to form plaques when plated on sensitive bacteria. If the phages are temperate, this inactivation means that they are no longer able to provoke the lytic response of the bacteria. As UV'd temperate phages do not kill the bacteria it is possible to imagine that they are provoking the lysogenic rather than the lytic response in the bacteria they have infected. If this were true, UV'd temperate phages plated on sensitive bacteria and, *at any time later*, given a further *inducing* dose of UV should show a larger number of survivors than if they had not been induced.

This is what happens for λ except that an hour after adsorption of the phages to the bacteria the "inducibility" has disappeared. Thus the UV'd phages did not form true lysogenic complexes. Another experiment points to the same conclusion: the virulent mutant of λ, which never forms lysogenics, also shows an increase in the number of plaques after irradiation of the complexes (UV'd virulent mutant-bacterium).

Thus the increase in the number of survivors by UV irradiation of the complexes (UV'd phage-bacterium) or by irradiation of the bacteria separately is a new type of reactivation. We shall call it UV restoration (UVR) in view of the fact that most of the experiments reported here were made with UV. It is possible that further experiments with x-rays and nitrogen mustard may show what is common to the action of these agents and allow a better name to be chosen to describe this reactivation.

The experiments have shown that the UV'd phages, in order to be UVR'd, must be adsorbed on UV'd bacteria. It is thus the bacteria that are reactivating and not a substance they might have released after irradiation.

The bacteria reactivate the UV'd phages under the following conditions: (1) When they have been treated with x-rays, with nitrogen mustard, or with UV. These three agents are inductors of lysogenic bacteria, they are mutagenic, and they disrupt the nuclear apparatus of the bacteria. It is

not known if these different actions are one aspect of the same cause, or which of these effects is responsible for reactivation. (2) The bacteria conserve the reactivating property for a long time when they are kept in buffer at 4°C. (3) The effect of UV on the bacteria, making them reactivating, can be reversed by exposure to visible light. (4) The reactivation as a function of the dose of UV given to the bacteria can be described by assuming that a proportion of the UV'd bacteria is in the reactivating state. This proportion is independent of the UV dose given to the phages. It is very approximately equal to the proportion of bacteria killed as colony formers.

Let us now sum up our findings concerning the properties of the reactivated phages: (1) The phage progeny liberated by a UV'd bacterium infected by a UV'd phage, consists of normal (except for mutations) viable phages which plate with equal efficiencies on both normal and irradiated bacteria. (2) UV'd phages plated on normal bacteria can be photoreactivated (PhR). If after the PhR the complexes are irradiated with UV, a further reactivation takes place. This is also true when UV irradiation has been given prior to illumination. This shows that the two classes of phages reactivable by visible light and by UV'd bacteria, respectively, are not identical. (3) After the double action of PhR and UVR a certain proportion of the UV'd phages remain inactivated, showing that certain UV damages cannot be repaired either by light or UV. (4) Mutants appear among the UVR'd phages. The action of UV on *both* the phages and the bacteria is necessary for their appearance. The mutation affects the entire progeny of the primary UV'd phage particle.

These facts can be accounted for by the following assumptions: UV irradiation produces four different types of lesions or damages in the phages. Type 1 lesions are not repairable by either UVR or PhR. Type 2 are repairable by either, type 3 by PhR only, and type 4 by UVR only.

Any one phage particle may have suffered damages of several of these types as a result of exposure to UV. A small fraction of the type 4 damages and *only of the damages of this type* are associated with mutations.

Our experiments do not tell us what is the specific role played by the action of the UV on the phages and on the bacteria, respectively. They rather emphasize that *both* actions are necessary. This double causation is very obvious in the present case. It raises the question whether mutagenesis in other organisms might not involve a similar double causation.

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*RELATIONS ON ITERATED REDUCED POWERS**

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In this note we present the generalization of the relations on iterated squares[1] to the case of iterated cyclic reduced powers of arbitrary prime period p. As in the case $p = 2$, the new relations are used to solve some particular problems.

Throughout this paper we will use the definitions and notation recently introduced by Steenrod.[2]

1. For any complex K and odd prime p, the cyclic reduced power operations are homomorphism $\mathcal{P}^s$, $(s = 0, 1, \ldots)$,

$$\mathcal{P}^s : H^q(K;\ Z_p) \to H^{q+2s(p-1)}(K;\ Z_p)$$

They satisfy the following properties: $\mathcal{P}^s f^* = f^* \mathcal{P}^s$, where f is a map of one complex into another; $\mathcal{P}^0$ = identity; if $q = \dim u$ is even, $\mathcal{P}^{q/2} u = u^p$ (in cup-product sense); $\mathcal{P}^s u = 0$ when $s > q/2$.

As in the case of squares, an *iterated* cyclic reduced power is a composition of two or more of the $\mathcal{P}^s$, e.g., $\mathcal{P}^r \mathcal{P}^s \mathcal{P}^t$.

Let δ^* be the coboundary operator associated with the exact coefficient sequence $0 \to Z \to Z \to Z_p \to 0$. Our main result is the following

THEOREM 1.1 *For all $0 \leq r < sp$ the iterated cyclic reduced powers satisfy the following set of relations*[3]

$$(1.2)\quad \mathcal{P}^r \mathcal{P}^s = \sum_{i=0}^{[r/p]} (-1)^{r+i} \binom{(s-i)(p-1)-1}{r-ip} \mathcal{P}^{r+s-i} \mathcal{P}^i,$$

$$(1.3)\quad \mathcal{P}^r \delta^* \mathcal{P}^s = \sum_{i=0}^{[r/p]} (-1)^{r+i} \binom{(s-i)(p-1)}{r-ip} \delta^* \mathcal{P}^{r+s-i} \mathcal{P}^i + \sum_{i=0}^{[(r-1)/p]} (-1)^{r+i+1} \binom{(s-i)(p-1)-1}{r-ip-1} \mathcal{P}^{r+s-i} \delta^* \mathcal{P}^i, \qquad (\text{mod } p),$$

where $\binom{\quad}{\quad}$ *denotes the binomial coefficient with the usual conventions.*

An induction argument based on (1.2) proves the following

THEOREM 1.4. *The set* $\{\mathcal{P}^{p^k}\}$ *with* $k = 0, 1, \ldots$, *form a base in the sense that any other* $\mathcal{P}^r$ *can be expressed as a sum of iterated cyclic reduced powers with exponents powers of* p.

For example,

$$\mathcal{P}^r = \frac{1}{r!}(\mathcal{P}^1)^r \qquad \text{for } 0 < r < p.$$

$$\mathcal{P}^{2p} = \frac{1}{2}(\mathcal{P}^p)^2 + \frac{1}{2}(\mathcal{P}^1)^{p-1}\mathcal{P}^p\mathcal{P}^1$$

where $(\mathcal{P}^j)^r$ means $\mathcal{P}^j$ iterated r times.

Again, an induction using formula (1.2) proves

THEOREM 1.5. *The iterated powers of the type* $\mathcal{P}^{i_1} \ldots \mathcal{P}^{i_r}$ *with* $i_1 \geq pi_2, \ldots, i_{r-1} \geq pi_r$ *and* $c = i_1 + \ldots + i_r$, *form an additive base for all iterated powers* $\mathcal{P}^{j_1} \ldots \mathcal{P}^{j_n}$ *where* $j_1 + \ldots + j_n = c$.

2. For the particular values $r = 1$, $s = 2^k - 1$, formula (1.2) becomes

$$\mathcal{P}^1\mathcal{P}^{2^k-1} = 2^k\mathcal{P}^{2^k},$$

therefore, if $\dim u = 2^{k+1}$ we have

$$u^p = \frac{1}{2^k}\mathcal{P}^1\mathcal{P}^{2^k-1}u \qquad (\text{mod } p), \tag{2.1}$$

where u^p is the p-power of u in the cup-product sense.

Let $H(K)$ denote the integral cohomology ring of a complex K. We say that $H(K)$ is a truncated polynomial ring on u if $H(K)$ is generated by the cup-product powers of u and each power is of infinite order. The height of u is the minimal integer n such that $u^n = 0$.

THEOREM 2.2. *If* $H(K)$ *is a non-trivial truncated polynomial ring on* u *then* $\dim u = 2^{k+1}$. *Moreover, if* $\dim u \geq 8$ *then the height of* u *is at most 3.*

We will show how this theorem is implied by our relations on reduced powers. Let $q = \dim u$. First, that q cannot be odd follows from the commutative law for cup-products. Now, if q is not a power of 2, then, because $\{\mathrm{Sq}^{2^k}\}$ is a base for squares,[1] we have $u \smile u = \mathrm{Sq}^q u = 0 \pmod 2$, and this is a contradiction. Finally, suppose $q = 2^{k+1}$. Using (2.1) for $p = 3$, we have

$$u \smile u \smile u = (-1)^k\mathcal{P}^1\mathcal{P}^{2^k-1}u \qquad (\text{mod } 3),$$

and $\dim \mathcal{P}^{2^k-1}u = 3\cdot 2^{k+1} - 4$. Therefore $u \smile u \smile u = 0 \bmod 3$, unless $3\cdot 2^{k+1} - 4$ is a multiple of 2^{k+1}. That is the case only if $k = 0, 1$.

Let S^{r-1} be a sphere bundle with S^{s-1} as fiber. Examples are known for the following forms of r and s: $r = s$; all r, $s = 1$; $r = 2n$, $s = 2$; $r = 4n$, $s = 4$; $r = 16$, $s = 8$.

COROLLARY 2.3. *The other possible values of r and s for which S^{r-1} can be a sphere bundle with fiber S^{s-1} are of the form $r = 2^{k+1}$ and $s = 2^k$, $(k \geq 4)$.*

Proof: If S^{r-1} is fibered by S^{s-1}, it follows from Gysin's sequence for sphere bundles that the integral cohomology ring of the base space B is a truncated polynomial ring, generated by the characteristic class u of dimension s. Then $r = ns$ for some integer n and dim $B = s(n-1)$; therefore the height of u is n. If $n > 3$, then 2.3 follows from 2.2. If $n = 3$ and $f: S^{r-1} \to B$ is the projection, adjoin an r cell E^r to B by means of f, so that $M = B \cup e^r$ is a manifold. By duality $H(M)$ is a truncated polynomial ring generated by u with height 4. This contradicts 2.2.

3. Our proof of relations (1.2), (1.3) is purely algebraic. The relations are obtained as homology relations on the symmetric group S_{p^2} of degree p^2, and makes full use of the general definition for reduced power operations found recently by Steenrod.[2] We will indicate briefly this method. Let G be a p-sylow group of S_{p^2} and $\theta: G \to S_{p^2}$ the inclusion homomorphism. For each $C \in H_i(G; Z_p)$ we have a reduced power operation. If $u \in H^q(K; Z_p)$ then $u^{p^2}/C \in H^{p^2q-i}(K; Z_p)$.

To obtain the relations we first identify the operations induced by some cycles of $H_i(G; Z_p)$ with sums of cyclic reduced powers. The relations are then obtained, according to the general principle of Steenrod,[2] as elements on the kernel of $\theta_*: H_i(G; Z_p) \to H_i(S_{p^2}; Z_p)$, i.e., if $\theta_*(C_1 - C_2) = 0$, then $u^{p^2}/C_1 = u^{p^2}/C_2$.

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[1] Adem, J., these PROCEEDINGS, **38**, 720–726 (1952).

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[3] I have heard that H. Cartan has obtained relations of the same type, using methods quite different from mine.

CONSTRAINED GAMES AND LINEAR PROGRAMMING

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Although Dresher, Karlin, and Shapley[1, 2] have also made clear their fundamental importance for "polynomial" infinite two-person zero-sum games, constrained games are usually considered to arise[3] when the mixed strategies of a finite rectangular (or "matrix") game are subjected to further linear inequalities. Adopting the finite view, the constrained games might alternatively be considered a "semi-normalization" of an unconstrained game in the sense of presentation of the game in a form part way to normalization, e.g., the combination could be reduced to an equivalent rectangular game in normal form by introducing pseudo "pure" strategies corresponding to the extreme points of the convex polyhedra of the restricted mixed strategies. In this new sense, the constrained game is also one in which some entries in the pay-off matrix of the normal form (and corresponding "pure" strategies) are only given implicitly in terms of other entries (and pure strategies).

Such constrained games permit one more realistic and convenient formulation of statistical, economic, and military decision problems,[3] and even in Borel's idea of games to evaluate habits as well as "skills" of players.[4] The above mentioned reduction to normal form is evidently an "in principle only" device, except for small or trivial problems as is also the ingenious fixed-point method.[1, 2] A sufficiently direct convenient and powerful vehicle for analysis and computation is required.

This note provides immediate transcription into a "linear programming" problem.[5, 6] The equivalence proof is an extremely brief proof of the fundamental theorem of zero-sum two-person *constrained* games—which extends directly to "convex" games[2]—and which does not require fixed-point theory. Transcript and proof exhibit directly a saddle-point to the expected pay-off function as an optimal pair of solutions of the programming problem and its dual (hence an immediate characterization of the "value")—and without variable changes or pay-off entry adjustments.[7, 8] Particular applications and other extensions will be dealt with elsewhere.

Proof: Consider the dual linear programming problems:

I			II		
(1a)	$\sum_i u_i a_{ij} + \mu + \sum_r v_r b_{rj}$	≥ 0	(1b)	$\sum_j a_{ij} x_j - \lambda + \sum_s y_s d_{is}$	≤ 0
(2a)	$\sum_i u_i$	$= 1$	(2b)	$\sum_j x_j$	$= 1$
(3a)	$\sum_i u_i d_{is}$	$\geq d_s$	(3b)	$\sum_j b_{rj} x_j$	$\leq b_r$
	minimize $\mu + \sum_r v_r b_r$			maximize $-\lambda + \sum_s y_s d_s$	

where $u_i, v_r, x_j, y_s \geq 0$, μ and λ are unrestricted in sign, and all summation ranges are finite and fixed by the index. The a_{ij} are pay-off matrix entries (second player's payment to first), the u_i, x_j are, respectively, mixed strategy frequencies of first and second player; the constraints are specified by the (resp.) third inequation. (Note that *each* of these problems contains *all* essential constants.) Conditions (3a) and (3b) are, respectively, assumed consistent.

The following chains of inequalities on the expected pay-off, $\sum_i \sum_j u_i a_{ij} x_j$, immediately result on multiplying and summing in the (resp.) first inequation sets, noting the second and then applying the constraint conditions:

$$-\mu - \sum_r v_r b_r \leq -\mu - \sum_j \sum_r v_r b_{rj} x_j \leq \sum_i \sum_j u_i a_{ij} x_j \qquad (4a)$$

$$\sum_i \sum_j u_i a_{ij} x_j \leq \lambda - \sum_i \sum_s y_s d_{is} u_i \leq \lambda - \sum_s y_s d_s \qquad (4b)$$

By (4*a*), (4*b*), and the apparent boundedness of the x_j, u_i sets, the respective functionals have finite maxima and minima. The dual theorem of Tucker, Kuhn, and Gale[7] asserts their equality. Letting asterisks on quantities connote their being part of an optimal solution, then for any pair by (4a), (4b)

$$-\mu^* - \sum_r v_r^* b_r = \sum_i \sum_j u_i^* a_{ij} x_j^* = \lambda^* - \sum_s y_s^* d_s \qquad (5)$$

(hence existence and characterization of a "value")

$$\sum_i \sum_j u_i^* a_{ij} x_j^* = -\mu^* - \sum_r v_r^* b_r \leq \sum_i \sum_j u_i^* a_{ij} x_j \qquad (5a)$$

$$\sum_i \sum_j u_i a_{ij} x_j^* \leq \lambda^* - \sum_s y_r^* d_r = \sum_i \sum_j u_i^* a_{ij} x_j^* \qquad (5b)$$

i.e.,

$$\sum_i \sum_j u_i a_{ij} x_j^* \leq \sum_i \sum_j u_i^* a_{ij} x_j^* \leq \sum_i \sum_j u_i^* a_{ij} x_j \qquad (6)$$

Thus, any optimal pair u_i^*, x_j^* yields a saddle-point as asserted.

For most computation (or analysis of constraint effects) it suffices to deal with merely one of I, II since (1) a "simplex" computation automatically yields a pair of dual optimal solutions, and (2) the "regrouping principle"[9] can generally be used to complete an optimal pair from one known member. All game solutions may then be obtained.[5]

If the constraints restrict the (resp.) mixed strategies to general compact convex sets ("convex" game[2]), then on approximating by inscribed monotone increasing polyhedral sets, compactness insures a limit point pair which sub-sequence convergence (of points and sets) guarantees is a saddle-point—thus a fixed-point-free convex games proof with constructively useful approximation.

Acknowledgment.—The author thanks W. W. Cooper for discussion and A. W. Tucker for suggestive lecture notes.

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ON ARITHMETIC GENERA OF ALGEBRAIC VARIETIES

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1. Introduction.—There are two distinct ways in which arithmetic genera of algebraic varieties may be defined. Let M_n be an irreducible non-singular algebraic variety of dimension n imbedded in a complex projective space $\mathfrak{S}$ and let E be a general hyperplane section of M_n. Then, for an arbitrary divisor D on M_n, there exists a polynomial $v(h; D)$ in h of degree n such that

$$\dim |D + hE| = v(h, D), \qquad \text{for large } h,$$

where $|D + hE|$ denotes the complete linear system on M_n consisting of all effective divisors which are linearly equivalent to $D + hE$. Moreover, the value $v(0, D)$ of this polynomial at $h = 0$ does not depend on the choice of the ambient space $\mathfrak{S}$ and is determined uniquely by D and M_n. We call $v(0; D)$ the virtual dimension of the complete linear system $|D|$. Now, in the first place, we may define the arithmetic genus[1] $P_a(M_n)$ of M_n to be the virtual dimension of the canonical system $|K|$ on M increased by $1 - (-1)^n$:

$$P_a(M_n) = v(0; K) + 1 - (-1)^n. \tag{1}$$

Alternatively the arithmetic genus[2] $p_a(M_n)$ of M_n may be defined by

$$p_a(M_n) = (-1)^n v(0;\ 0), \tag{2}$$

where the second "0" in $v(0;\ 0)$ denotes the divisor zero on M_n. It is known[3] that the equality $p_a(M_n) = P_a(M_n)$ holds in case $n = 1$ or 2. For $n = 3$, Severi[4] has established the equality $p_a(M_3) = P_a(M_3)$ by making use of the theorem of completeness of the characteristic system of a complete continuous system of surfaces on M_3, whereas no proof of this theorem of completeness is known. A proof of the equality $p_a(M_3) = P_a(M_3)$ following the lines of Severi and based on the theory of harmonic integrals has been given recently by one of the authors.[5] On the other hand, Zariski has shown in his recent paper[6] that the equality $p_a(M_n) = P_a(M_n)$ can be derived easily from $p_a(M_{n-1}) = P_a(M_{n-1})$ *in case* n *is even*. Hence the equality $p_a(M_4) = P_a(M_4)$ follows from $p_a(M_3) = P_a(M_3)$. For $n \geqq 5$, the equality $p_a(M_n) = P_a(M_n)$ has not yet been established rigorously so far as we know, without special assumptions.[7] The main purpose of the present note is to prove the equality $p_a(M_n) = P_a(M_n)$ in full generality by means of the theory of faisceaux.

2. *Virtual Arithmetic Genera.*—Denoting by $g_s(M_n)$ the number of linearly independent s-pl differentials of the first kind on M_n, we set

$$a(M_n) = \sum_{s=0}^{n} (-1)^s g_s(M_n). \tag{3}$$

Then, as was shown by one of the authors,[8] the equality

$$P_a(M_n) = (-1)^n\{a(M_n) - 1\} \tag{4}$$

holds. In view of this equality, we also call $a(M_n)$ the arithmetic genus of M_n. Now the *virtual arithmetic genus* $a_M(D)$ of an arbitrary divisor D on $M = M_n$ is defined as follows:[9] Let D_h be a general member of the complete linear system $|D + hE|$, E being a general hyperplane section of M_n. Then, for sufficiently large h, D_h is a non-singular subvariety of M_n and therefore $a(D_h)$ is well defined (in case $n = 1$, D_h is a divisor composed of simple points only; then we *define* $a(D_h)$ to be the degree of D_h). Moreover there exists a polynomial $a(h;\ D,\ M_n)$ such that $a(D_h) = a(h;\ D,\ M_n)$ for large h. By means of this polynomial, we define $a_M(D)$ by

$$a_M(D) = a(0;\ D,\ M_n).$$

It can be shown that the functional $a_M(D)$ thus defined is independent of the choice of the ambient space $\mathfrak{Z}$ of M and has the following properties:[10] (i) *in case* $D = S$ *is a non-singular subvariety of* M, *we have* $a_M(S) = a(S)$; (ii) *if* $D \approx D'$ *on* M, *then we have* $a_M(D) = a_M(D')$, *where* $\approx$ *denotes linear equivalence;* (iii) *the equality*

$$a_M(D + S) = a_M(D) + a(S) - a_S(D \cdot S) \tag{5}$$

holds, where $D \cdot S$ *is the divisor on the non-singular subvariety* S *cut out by* D.

Now, using the theorem of Riemann-Roch for adjoint systems, we infer readily from (2) that $p_a(M_n)$ may be expressed in the form[11]

$$p_a(M_n) = a(M_n) - a_M(-K) - (-1)^n,$$

while $P_a(M_n)$ is given by (4). In order to prove the equality $p_a(M_n) = P_a(M_n)$, it is sufficient therefore to show that[12]

$$a_M(-K) = [1 - (-1)^n]a(M_n). \tag{6}$$

3. Euler Characteristics of Compact Analytic Manifolds with Respect to Faisceaux.—Let $M = M_n$ be a compact complex analytic manifold of dimension n. Given a complex analytic faisceau[13] $\mathfrak{F}$ on M, we introduce the corresponding *Euler characteristic*

$$\chi(M, \mathfrak{F}) = \sum_{s=0}^{n} (-1)^s \dim H^s(M, \mathfrak{F}),$$

where $H^s(M, \mathfrak{F})$ denotes the s-cohomology of M with coefficients in $\mathfrak{F}$, provided that $\mathfrak{F}$ satisfies the condition

$$\dim H^s(M, \mathfrak{F}) \begin{cases} < +\infty, & \text{for } 0 \leqq s \leqq n, \\ = 0, & \text{for } s > n. \end{cases} \tag{F}$$

Now, letting $\Omega^r = \Omega^r(M)$ be the faisceau of germs of holomorphic r-forms on M, we set

$$\chi^r(M) = \chi(M, \Omega^r),$$

provided that Ω^r satisfies the condition (F).

Let $A^{r,s}$ be the faisceau of germs of currents of type (r, s) on M, $F^{r,s}$ the subfaisceau of $A^{r,s}$ formed by the germs which are closed under $\bar{\partial}$. We have the exact sequence

$$0 \longrightarrow F^{r,s} \xrightarrow{i} A^{r,s} \xrightarrow{\bar{\partial}} F^{r,s+1} \longrightarrow 0,$$

i being the inclusion map, and, from the corresponding exact cohomology sequence, we obtain the canonical isomorphism[14]

$$H^s(M, F^{r,t}) \cong H^{r,t+s}(M)$$

where

$$H^{r,t+s}(M) = \Gamma(M, F^{r,t+s})/\bar{\partial}\Gamma(M, A^{r,t+s-1})$$

is the $\bar{\partial}$-cohomology of currents of type $(r, s + t)$, $\Gamma(M, F)$, $\Gamma(M, A)$ being the vector spaces of sections. If we take $t = 0$ and observe that $F^{r,0} = \Omega^r$, we have therefore

$$H^s(M, \Omega^r) \cong H^{r,s}(M). \tag{7}$$

If M is a compact Kähler manifold, then $H^{r,s}(M)$ is (canonically isomor-

phic to) the linear space of harmonic forms of type (r, s) on M. This shows that Ω^r satisfies the condition (F). Thus the Euler characteristics $\chi^r(M)$ are well defined for any compact Kähler manifold M. In particular, since $\dim H^{0,s}(M) = \dim H^{s,0}(M) = g_s(M)$ and $\dim H^{n,s}(M) = \dim H^{n-s,0}(M) = g_{n-s}(M)$, we have, by (3),

$$\chi^0(M) = a(M), \tag{8}$$

$$\chi^n(M_n) = (-1)^n a(M_n) \tag{9}$$

for any compact Kähler manifold $M = M_n$.

Let D be a divisor on M and, for each point p on M, let $f_p(D)$ be the germ of a meromorphic function expressing the divisor D at p. Then we say that a germ τ_p of a meromorphic r-form is a multiple of $-D$ if $f_p(D) \cdot \tau_p$ is holomorphic at p. Now, letting $\Omega^r_D = \Omega^r_D(M)$ be *the faisceau of germs of meromorphic r-forms which are multiples of* $-D$, we define the corresponding Euler characteristic

$$\chi^r_D(M) = \chi(M, \Omega^r_D)$$

provided that Ω^r_D satisfies the condition (F).

First, we consider the case in which $D = S$ is a non-singular subvariety of M of dimension $n - 1$. For each point $p \in S$ we can introduce local analytic coordinates $z^1_p, \ldots, z^n_p$ with the center p such that $z^1_p = 0$ on S and $z^2_p, \ldots, z^n_p$ are local analytic parameters on S. Let $z^i_p = \bar{z}_p{}^{i-n}$ for $i = n + 1, \ldots, 2n$. Given an arbitrary s-form φ defined in a neighborhood of p,

$$\varphi = \sum_{i_1 < \ldots < i_s} \varphi_{i_1 \ldots i_s} dz^{i_1}_p \ldots dz^{i_s}_p,$$

we set

$$\nu_p \cdot \varphi = \sum_{1 < i_2 < \ldots < i_s} \varphi_{1 i_2 \ldots i_s} dz^{i_2}_p \ldots dz^{i_s}_p.$$

Now let τ_p be a meromorphic r-form defined in a neighborhood U_p of the point $p \in S$, and suppose that τ_p is a multiple of $-S$—that is, we suppose that $z^1_p \tau_p$ is holomorphic in U_p. Then τ_p is a current in U_p of type $(r, 0)$. Given any form ψ of type $(n - r, n - 1)$ and of class C^∞ whose carrier is a compact subdomain of U_p, an easy calculation shows that

$$\delta\tau_p[\psi] = 2\pi i \int_S \{\nu_p(z^1_p \tau_p) \cdot \psi + (-1)^r (z^1_p \tau_p) \cdot \nu_p \psi\}.$$

If $p, q \in S$, $U_p \cap U_q$ is not empty, we set $h_{pq} = (z^1_p / z^1_q)_S$ in $U_p \cap U_q$ where $(z^1_p / z^1_q)_S = z^1_p / z^1_q | S$ is the restriction of the ratio z^1_p / z^1_q to S. The system $\{h_{pq}\}$ defines a principal fiber bundle over S with fiber the multiplicative group $\mathbf{C}^*$ of complex numbers. Let the fiber $\mathbf{C}^*$ in this bundle be replaced by the complex projective line $\mathfrak{S}_1$, and denote the resulting bundle by $\mathfrak{B}$. If $\mathfrak{B}$ has an analytic cross-section defined by a system $\{h_p\}$ of meromorphic functions h_p defined, respectively, in U_p, $h_p / h_q = h_{pq}$ in $U_p \cap U_q$, we denote

by C the divisor on S which is in U_p given by $C = (h_p)$, where (h_p) denotes the divisor of h_p. The divisor $\mathcal{C}$ is called the *characteristic divisor* of the subvariety S of M. We suppose that C exists; it always exists if M is an algebraic variety.[15]

Let $\mathfrak{P}(\tau_p) = (\nu_p \cdot z_p^1 \tau_p)_S$, $\mathfrak{Q}(\tau_p) = (z_p^1 \tau_p)_S / h_p$. We call $\mathfrak{P}(\tau_p)$, $\mathfrak{Q}(\tau_p)$, respectively, the *Poincaré residue*, the *Severi residue* of τ_p. Setting

$$\mathfrak{R}_S(\tau_p)[\psi] = \int_S \{\mathfrak{P}(\tau_p) \cdot \psi + (-1)^r \mathfrak{Q}(\tau_p) h_p \cdot \nu_p \psi\}, \tag{10}$$

we have the formula

$$\bar{\partial} \tau_p[\psi] = 2\pi i \mathfrak{R}_S(\tau_p)[\psi]. \tag{11}$$

The current $\mathfrak{R}_S(\tau_p)$ is called the residue of τ_p on S.

Let $\mathfrak{R}_S^r$ be the residue faisceau which is obtained from Ω_S^r by applying the operator $\bar{\partial}$. Then we have the exact sequence

$$0 \to \Omega^r \xrightarrow{i} \Omega_S^r \to \mathfrak{R}_S^r \to 0 \tag{12}$$

where i is the inclusion map. Moreover we have the canonical isomorphism

$$\mathfrak{R}_S^r \cong \Omega^{r-1}(S) + \Omega_C^r(S). \tag{13}$$

In fact, $\mathfrak{P}(\tau_p) \in (\Omega^{r-1}(S))_p$, $\mathfrak{Q}(\tau_p) \in (\Omega_C^r(S))_p$ for each $\tau_p \in (\Omega_S^r)_p$; conversely, given $\omega_1 \in (\Omega^{r-1}(S))_p$, $\omega_2 \in (\Omega_C^r(S))_p$,

$$\tau_p = d \log z_p^1 \cdot \omega_1 + h_p \omega_2 / z_p^1$$

belongs to $(\Omega_S^r)_p$ and satisfies $\mathfrak{P}(\tau_p) = \omega_1$, $\mathfrak{Q}(\tau_p) = \omega_2$. Hence we infer from (10), (11) the isomorphism (13).

The exact cohomology sequence corresponding to (12) is

$$\cdots \longrightarrow H^s(M, \Omega^r) \xrightarrow{i^*} H^s(M, \Omega_S^r) \xrightarrow{\bar{\partial}^*} H^s(M, \mathfrak{R}_S^r) \xrightarrow{\delta^*} H^{s+1}(M, \Omega^r) \longrightarrow \cdots.$$

We obtain from this the important formula[16]

$$\dim H^s(M, \Omega_S^r) = \dim H^s(M, \mathfrak{R}_S^r) - \dim H^{s+1}(M, \Omega^r) + \dim \{H^s(M, \Omega^r)/\delta^* H^{s-1}(M, \mathfrak{R}_S^r)\} + \dim \{H^{s+1}(M, \Omega^r)/\delta^* H^s(M, \mathfrak{R}_S^r)\}, \tag{14}$$

where, in view of (13),

$$\dim H^s(M, \mathfrak{R}_S^r) = \dim H^s(S, \Omega^{r-1}) + \dim H^s(S, \Omega_C^r) \tag{15}$$

Now, multiplying both sides of (14) by $(-1)^s$ and adding on s from 0 to n, we obtain the formula

$$\chi_S^r(M) = \chi^r(M) + \chi^{r-1}(S) + \chi_C^r(S), \tag{16}$$

provided that each faisceau Ω^r, Ω_S^r, $\mathfrak{R}_S^r$ satisfies the condition (F).

Assume now that M is a compact Kähler manifold and consider the case in which $r = n$. In this case the faisceau $\Omega_C^n(S)$ vanishes and therefore the canonical isomorphism (13) is reduced to

$$\mathfrak{O}_S^n \cong \Omega^{n-1}(S), \tag{13'}$$

while S has the Kähler structure induced by the imbedding in M. Hence, by (7), Ω^n, $\mathfrak{O}_S^n$ both satisfy (F), and therefore, as one readily infers from (14), Ω_S^n also satisfies (F). Thus we obtain from (16) and (9) the formula

$$a(S) = (-1)^{n-1}\{\chi_S^n(M_n) - \chi^n(M_n)\} \tag{18}$$

for an arbitrary non-singular variety S of dimension $n-1$ of the compact Kähler manifold M_n of dimension n.

4. A Definition of Virtual Arithmetic Genera in Terms of Faisceaux.—In what follows we assume $M = M_n$ to be a non-singular algebraic variety imbedded in a projective space. The formula (18) suggests that we may define the virtual arithmetic genus of an arbitrary divisor D on M by

$$\bar{a}(D) = (-1)^{n-1}\{\chi_D^n(M) - \chi^n(M)\}. \tag{19}$$

In this section we first show that the right-hand side of (19) is well defined, that is, that the faisceau Ω_D^n satisfies the condition (F), and then prove that $\bar{a}(D)$ coincides with the virtual arithmetic genus $a_M(D)$. For this purpose we remark that the linear equivalence $D \approx D'$ implies the isomorphism $\Omega_D^n = \Omega_{D'}^n$. In fact, letting f be the meromorphic function on M with the divisor $(f) = D' - D$, we infer that $\tau_p \to \tau_p' = f \cdot \tau_p$ induces the isomorphism $\Omega_D^n \cong \Omega_{D'}^n$. It follows that

$$H^s(M, \Omega_D^n) \cong H^s(M, \Omega_{D'}^n), \qquad \text{if } D \approx D' \text{ on } M. \tag{20}$$

Let S be a non-singular subvariety of M of dimension $n-1$ which is not a component of D. Then we have the exact sequence

$$0 \longrightarrow \Omega_D^n \xrightarrow{i} \Omega_{D+S}^n \xrightarrow{\mathfrak{P}} \Omega_{DS}^{n-1}(S) \longrightarrow 0, \tag{21}$$

where $\mathfrak{P}$ is the mapping $\tau_p \to \mathfrak{P}(\tau_p) = (\nu_p \cdot z_p^1 \tau_p)_S$ introduced in section 3. In case $n = 1$, we have the exact sequence

$$0 \longrightarrow \Omega_D^1 \xrightarrow{i} \Omega_{D+S}^1 \xrightarrow{\mathfrak{P}} \Omega^0(S) \longrightarrow 0 \tag{21'}$$

for any divisor $S = \sum_{k=1}^{m} p_k$ with simple components only, where $\Omega^0(S)$ is the linear space of complex valued functions defined on the finite set $S = \{p_1, p_2, \ldots, p_m\}$. The exact cohomology sequence corresponding to (21) is

$$\ldots \longrightarrow H^s(M, \Omega_D^n) \xrightarrow{i^*} H^s(M, \Omega_{D+S}^n) \xrightarrow{\mathfrak{P}^*} H^s(S, \Omega_{DS}^{n-1}(S)) \xrightarrow{\delta^*} H^{s+1}(M, \Omega_D^n) \longrightarrow \ldots. \tag{22}$$

In order to prove that Ω_D^n satisfies (F), let $S = E_h$ be a general hypersurface section of M of sufficiently high order h. Then the general member T of the complete linear system $|D + S|$ is a non-singular subvariety of M of dimension $n - 1$ and $H^s(M, \Omega_{D+S}^n) \cong H^s(M, \Omega_T^n)$ by (20), while Ω_T^n satisfies (F), as was shown in Section 3. Hence Ω_{D+S}^n also satisfies (F). We infer, therefore, from (22) that Ω_D^n satisfies (F) if the faisceau $\Omega_{DS}^{n-1}(S)$ on the variety $S = E_h$ of dimension $n - 1$ satisfies the condition (F). Thus, by induction on n, our problem is reduced to the case in which $n = 1$. Now, in case $n = 1$, $S = E_h$ is a divisor with simple points only, that is $S = \sum_{k=1}^{m} p_k$, and the same argument as above shows that Ω_D^1 satisfies (F) if $\Omega^0(S)$ satisfies (F), while it is obvious that $\dim H^s(S, \Omega^0(S)) = m$ for $s = 0$, and is equal to 0 for $s \geqq 1$. Hence Ω_D^1 satisfies (F).

Thus Ω_D^n satisfies the condition (F) and therefore we obtain from (22) the equality

$$\chi_{D+S}^n(M) = \chi_D^n(M) + \chi_{DS}^{n-1}(S). \tag{23}$$

In case $n = 1$ we have furthermore

$$\chi_{D+S}^1(M) = \chi_D^1(M) + m, \qquad \text{for } S = \sum_{k=1}^{m} p_k. \tag{23'}$$

It follows from (23) that

$$\tilde{a}(D + S) = \tilde{a}(D) + a(S) - \tilde{a}(D \cdot S). \tag{24}$$

In fact, we get, using (9),

$$\tilde{a}(D + S) = (-1)^{n-1}\{\chi_{D+S}^n(M) - \chi^n(M)\} = (-1)^{n-1}\{\chi_D^n(M) - \chi^n(M) + \chi_{DS}^{n-1}(S) - \chi^{n-1}(S)\} + (-1)^{n-1}\chi^{n-1}(S) = \tilde{a}(D) + a(S) - \tilde{a}(D \cdot S).$$

In case $n = 1$, we obtain from (23)'

$$\tilde{a}(D + S) = \tilde{a}(D) + m, \qquad \text{for } S = \sum_{k=1}^{m} p_k. \tag{24'}$$

Incidentally it follows from (20) that

$$\tilde{a}(D) = \tilde{a}(D') \qquad \text{if } D \approx D' \text{ on } M. \tag{25}$$

Now we prove the equality $\tilde{a}(D) = a_M(D)$ by induction on n. In case $n = 1$, any divisor D is linearly equivalent to a divisor of the type $S - T$, $S = \sum_{k=1}^{m} p_k$, $T = \sum_{k=1}^{l} q_k$, and therefore $\tilde{a}(D) = \tilde{a}(S - T) = \tilde{a}(S) - l$ by (25) and (24'), while (24') implies that $\tilde{a}(S) = m$. Hence we obtain $\tilde{a}(D) =$ degree of $D = a_M(D)$. Now, assume the equality $\tilde{a}(D) = a_{M_{n-1}}(D)$ for the variety M_{n-1} of dimension $n - 1$ and consider $M = M_n$ of dimen-

sion $n \geqq 2$. Let $S = E_h$ be a general hypersurface section of sufficiently high order h and let T be a general member of $|D + S|$. Then we get, by (24) and (25)

$$\tilde{a}(T) = \tilde{a}(D) + a(S) - \tilde{a}(D \cdot S),$$

while

$$a(T) = a_M(D) + a(S) - a_S(D \cdot S),$$

as the results given in section 3 show. Now, by (18), we have $\tilde{a}(T) = a(T)$ for the non-singular variety T, while $\tilde{a}(D \cdot S) = a_S(D \cdot S)$ by the inductive hypothesis. Consequently we obtain $\tilde{a}(D) = a_M(D)$. Thus we have proved the formula

$$a_M(D) = (-1)^{n-1}\{\chi_D^n(M) - \chi^n(M)\}. \tag{26}$$

5. *Proof of the Equality $p_a = P_a$.*—As was shown in section 2, it is sufficient for our purpose to prove the formula (6). Let w be the meromorphic n-ple differential with the divisor $(w) = K$. Then the mapping $f_p \rightarrow \tau_p = f_p \cdot w$, $f_p \in (\Omega^\circ)_p$, $\tau_p \in (\Omega^n_{-K})_p$, induces the isomorphism $\Omega^\circ \cong \Omega^n_{-K}$, since $\tau_p = f_p \cdot w$ implies $(\tau_p) - K = (f_p)$. Consequently we have $H^s(M, \Omega^n_{-K}) = H^s(M, \Omega^\circ)$ and hence

$$\chi^n_{-K}(M) = \chi^\circ(M).$$

Using (26), (8) and (9), we get therefore

$$a_M(-K) = (-1)^{n-1}\{\chi^\circ(M) - \chi^n(M)\} = [1 - (-1)^n]a(M).$$

This proves the formula (6), q. e. d.

6. *The Birational Invariance of the Arithmetic Genus p_a.*—Combining the equality $p_a = P_a$ with (4), we obtain

$$p_a(M_n) = g_n(M_n) - g_{n-1}(M_n) + - \ldots + (-1)^{n-1}g_1(M_n),$$

while each $g_s(M_n)$ is known to be a birational invariant[17] of M_n. Hence we conclude that *the arithmetic genus $p_a(M_n)$ of a non-singular algebraic variety M_n is a birational invariant of M_n.*

[1] Severi, F., "Fondamenti per la geometria sulla varietà algebriche," *Rend. Circ. Mat. Palermo*, **28**, 33–87 (1909); Zariski, O., "Complete Linear Systems on Normal Varieties and a Generalization of a Lemma of Enriques-Severi," *Ann. Math.*, **55**, 552–592 (1952) (especially Part III). Compare also Kodaira, K. (1) "The Theory of Harmonic Integrals and Their Applications to Algebraic Geometry," Notes, Princeton University, 1953, p. 42; (2) "Some Results in the Transcendental Theory of Algebraic Varieties," *Ann. Math.* (to appear).

[2] See ref. 1.

[3] Zariski, *loc. cit.*, pp. 589–590.

[4] Severi, *loc. cit.*, pp. 52–56.

[5] Kodaira, K., "The Theorem of Riemann-Roch for Adjoint Systems on 3-Dimensional Algebraic Varieties," *Ann. Math.*, **56**, 298–342, §12 (1952).

[6] Zariski, *loc cit.*, pp. 591–592.

[7] A proof of the equality $p_a = P_a$ given by one of the authors [PROC. NATL. ACAD. SCI., **38,** 527–533 (1952)] has a serious gap and it is valid only for a special class of algebraic varieties.

[8] Kodaira, *loc. cit.* in ref. 1: (1), pp. 42–43 and (2), Theorem 6.4.

[9] Kodaira, *loc. cit.* in ref. 1: (1), pp. 39–42 and (2), §6.

[10] Kodaira, *loc. cit.* in ref. 1: (1), pp. 35–42 and (2), §6.

[11] Kodaira, *loc. cit. in* ref. 1: (1), p. 43 and (2), formula (6.23).

[12] Compare this with Zariski's conjecture, Zariski, *loc. cit.*, p. 590, formulas (61), (61′).

[13] The notion of a faisceau is due to J. Leray, but a generalization of Leray's theory has recently been given by H. Cartan, Séminaire de Topologie algébrique, 1950–1951. For analytic faisceaux, see Séminaire H. Cartan, 1951–1952.

[14] Dolbeault, P., "Sur la cohomologie des variétés analytiques complexes," *Compt. rend., Paris,* **236,** 175–177 (1953).

[15] See, for example, Kodaira, *loc. cit.* in ref. 5, §11.

[16] Spencer, D. C. (1) "Cohomology and the Riemann-Roch Theorem," Notes, Princeton University, 1953; (2) Note with the same title, PROC. NATL. ACAD. SCI. **39,** 660–669 (1953).

[17] von der Waerden, B. L., "Birational Invariants of Algebraic Manifolds, *Acta Salmanticensia,* **II,** §6 (1947).

EXTENSION THEOREMS FOR SOLUTIONS OF IRREFLEXIVE RELATIONS

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In a preceding paper,[1] various sufficient conditions for the existence of solutions of irreflexive relations were obtained. In the present note, conditions sufficient to guarantee that a solution of a subsystem can be extended to a solution of a supersystem are presented. The definitions and notations of SOIR are presupposed here. Proofs will be omitted for lack of space.

1. If H is a subgraph of the graph G, then the graph obtained by adding to H all the arcs of G which join pairs of vertices of H will be termed the *juncture* of H (relative to G), and will be denoted by $\bar{H}$. H is termed a *conjunct* subgraph of G if $H = \bar{H}$.

Let G_0 be the graph of the subsystem $(\mathfrak{T}_0, \succ)$ of the system $(\mathfrak{T}, \succ)$ having the graph G, $\succ$ being an irreflexive binary relation. Then G_0 is a conjunct closed subgraph of G. Let V_0 be a given solution of G_0.

If G_0 is a conjunct subgraph of G and x is any vertex of G, let $D^{-1}(x, G - G_0)$ be the set of all vertices y of $G - G_0$ such that $y \succ x$. If X is a set of vertices of G, let $D^{-1}(X, G - G_0) = \bigcup_{x \in X} D^{-1}(x, G - G_0)$, and let

$D^{-n}(X, G - G_0) = D^{-1}(D^{-n+1}(X, G - G_0), G - G_0)$ for $n > 1$. By the *predecessor-set* of X relative to $G - G_0$ is meant the set $P(X, G - G_0) = \bigcup_{n=1}^{\infty} D^{-n}(X, G - G_0)$. By a *predecessor-sequence* $p(x_0, G - G_0)$ of x_0 relative to $G - G_0$ is meant a regression $x_0, x_1, x_2, \ldots$ of maximum length, finite or infinite, such that all its vertices except possibly x_0 itself are in $G - G_0$; that is, such that one vertex x_n is chosen from each set $D^{-1}(x_{n-1}, G - G_0)$ for $n > 0$, all x_n's being distinct. Let $p^*(x, G - G_0)$ be the set of all vertices of the predecessor-sequence $p(x_0, G - G_0)$ other than x_0 itself. A predecessor-sequence $p(x_0, G - G_0)$ is termed *trivial* if and only if $p^*(x_0, G - G_0)$ is empty. We have $P(x_0, G - G_0) = \cup p^*(x_0, G - G_0)$ for all predecessor-sequences $p(x_0, G - G_0)$ of x_0 relative to $G - G_0$. Note that the elements of the predecessor-set of x_0 or of a predecessor-sequence of x_0 are not necessarily ancestors of x_0, although every ancestor of x_0 belongs to at least one predecessor-sequence of x_0 (all relative to $G - G_0$). If $\succ$ is not asymmetric, then a source, which has no ancestor, may have non-trivial predecessor-sequences.

THEOREM 1. *Let G be the graph of the system $(\mathfrak{D}, \succ)$ and G_0 the graph of a subsystem $(\mathfrak{D}_0, \succ)$, and let V_0 be a solution of G_0. Suppose that: (1) All non-trivial predecessor-sequences $p(x_0, G - G_0)$, $x_0 \in G_0$, are either infinite, or, if finite, of odd length if $x_0 \in W_0 = \mathfrak{D}_0 - V_0$, and of even length if $x_0 \in V_0$; (2) $D(V_0, G) \frown D^{-2n}(V_0, G - G_0) = D(V_0, G) \frown D^{-2n+1}(W_0, G - G_0) = 0$ for all $n > 0$; (3) if $h > 0$ and $k > 0$ are of the same parity then $D^{-h}(V_0, G - G_0) \frown D^{-k}(W_0, G - G_0) = 0$, and if $h > 0$ and $k > 0$ are of different parity then $D^{-h}(V_0, G - G_0) \frown D^{-k}(V_0, G - G_0) = D^{-h}(W_0, G - G_0) \frown D^{-k}(W_0, G - G_0) = 0$; (4) every vertex of $G - G_0$ is in $P(\mathfrak{D}_0, G - G_0)$. Then*

$$V = V_0 \smile \left(\bigcup_{n=1}^{\infty} D^{-2n}(V_0, G - G_0)\right) \smile \left(\bigcup_{n=1}^{\infty} D^{-2n+1}(W_0, G - G_0)\right)$$

is a solution of G and an extension of V_0.

COROLLARY. *Let G be the graph of the system $(\mathfrak{D}, \succ)$ and G_0 the graph of a subsystem $(\mathfrak{D}_0, \succ)$, and let V_0 be a solution of G_0. Suppose that: (a) All non-trivial predecessor-sequences $p(x_0, G - G_0)$, $x_0 \in G_0$, are infinite, or, if finite, of odd length if $x_0 \in W_0 = \mathfrak{D}_0 - V_0$, and of even length if $x_0 \in V_0$; (b) no vertex of any $P(x_0, G - G_0)$, $x_0 \in G_0$, is adjacent to any vertex of G_0 other than x_0; and if x_0 and x'_0 are any two distinct vertices of G_0, then $P(x_0, G - G_0) \frown P(x'_0, G - G_0) = 0$; (c) no $P(x_0, G - G_0) \smile (x_0)$, $x_0 \in G_0$, contains an odd cycle modulo 2; (d) every vertex of $G - G_0$ is in $P(\mathfrak{D}_0, G - G_0)$. Then solutions of G which are extensions of V_0 exist.*

2. We suppose that every vertex of $G - G_0$ is in the predecessor-set $P(\mathfrak{D}_0, G - G_0)$. If H is any conjunct subgraph of G, and x is a vertex of

G, let $C^{-1}(x, H) = \bigcup_{n=0}^{\infty} D^{-n}(x, H)$; that is, $C^{-1}(x, H)$ is the set of all vertices y of H which chain-dominate x by means of a chain all the vertices of which, except possibly x, lie in H, together with x itself. If $y \in C^{-1}(x, H)$ and $x \in C^{-1}(y, H)$, $x \neq y$, then x and y are termed *cyclically related* relative to H. If $y \in C^{-1}(x, H)$ but not $x \in C^{-1}(y, H)$ then x is termed a *descendant* of y relative to H. A sequence $\{x_n\}$ of vertices of H is termed a *descending sequence* if x_{n+1} is a descendant of x_n for all n (except the last n if the sequence is finite), and if there exists no vertex y which is a descendant of all x_n. If a vertex x of H has no descendant relative to H then $C^{-1}(x, H)$ is termed an *inverse basic set* of H, and x is termed a *sink* of this inverse basic set. A subgraph H is termed *descendingly finite* if every descending sequence of H is finite. The same inverse basic set may contain more than one sink; all sinks of the same inverse basic set are cyclically related relative to H.

LEMMA 1. *If H is descendingly finite, then every vertex of H belongs to some inverse basic set of H.*

LEMMA 2. *If H is descendingly finite, no proper subset B of an inverse basic set A is an inverse basic set.*

By an *inverse basis* of H is meant a set S of vertices of H such that: (*a*) $x, y \in S$, $x \neq y$, implies x is not chain-dominated by y relative to H; (*b*) $y \in H \frown \mathfrak{D} - S$ implies that there exists a vertex $x \in S$ such that $y \in C^{-1}(x, H)$, that is, x is chain-dominated by y relative to H.

LEMMA 3. *Every descendingly finite closed subgraph H has an inverse basis.*

LEMMA 4. *If H has an inverse basis S and $b_i \in S$, then $C^{-1}(b_i, H)$ is an inverse basic set of H of which b_i is a sink.*

LEMMA 5. *Every inverse basis S of a descendingly finite closed subgraph H consists of one sink from each inverse basic set of H.*

A graph H is termed *completely descendingly finite* if all its closed subgraphs are descendingly finite. A sequence $\{x_n\}$ of distinct vertices of H is termed a *progression* of H if $x_n \succ x_{n+1}$ and $\mathrm{Cl}(x_n x_{n+1}) \subset H$ for all n (except the last if the sequence is finite). If all the progressions of H are finite, H is termed *progressively finite*.

LEMMA 6. *A necessary and sufficient condition that H be completely descendingly finite is that H be progressively finite.*

The proofs of Lemmas 1–6 are similar to the proofs of the correspondingly numbered lemmas of SOIR with $\prec$, source, ancestor, basis, basic set regression, etc., replaced by $\succ$, sink, descendant, inverse basis, inverse basic set, progression, etc., respectively.

3. Suppose now that $\mathrm{Cl}(G - G_0)$ is completely descendingly finite. Let G_0 have the solution V_0. Let[2] $W_{00} = D(V_0, G_0)$ and $W_0 = D(V_0, G) \smile D^{-1}(V_0, G - G_0)$. Let $G_{-1} = G - \mathrm{St}(G_0 \smile W_0)$. By Lemma 3,

G_{-1} has an inverse basis. Let V_{-1} be an inverse basis of G_{-1}. For every natural number $k \geqq 1$ let $W_{-k} = D(V_{-k}, G_{-k}) \smile D^{-1}(V_{-k}, G_{-k})$, let $G_{-k-1} = G - \mathrm{St}\left[\bigcup_{i=0}^{k} (V_{-i} \smile W_{-i})\right] = G_{-k} - \mathrm{St}(V_{-k} \smile W_{-k})$, and let V_{-k-1} be an inverse basis of G_{-k-1}.

LEMMA 7. *G_{-k-1} is a conjunct subgraph of G for all $k \geqq 0$.*

LEMMA 8. *For all $k \geqq 0$, $\bigcup_{i=0}^{k+1} V_{-i}$ is satisfactory (with respect to non-domination).*

It may happen that $G_{-n} = 0$ for no finite ordinal n, in which case we may let $G_{-\omega} = G - \mathrm{St}\left[\bigcup_{i<\omega} (V_{-i} \smile W_{-i})\right]$, $V_{-\omega}$ = any inverse basis of $G_{-\omega}$, and $W_{-\omega} = D(V_{-\omega}, G_{-\omega}) \smile D^{-1}(V_{-\omega}, G_{-\omega})$, and so on. Transfinite induction shows that if β is an ordinal number for which $V_{-\alpha}$ is non-empty for all $\alpha < \beta$ then $\bigcup_{\alpha<\beta} V_{-\alpha}$ is satisfactory. Let the cardinal number of the set $\mathfrak{D}$ be $\aleph_\mu$. Let λ be the next largest ordinal after those of $\mathfrak{Z}(\aleph_\mu)$, where $\mathfrak{Z}(\aleph_\mu)$ is the set of all ordinal numbers of well-ordered sets having cardinal number $\aleph_\mu$. Then no matter how we well-order the elements of $\mathfrak{D}$, its ordinal number is $<\lambda$. Well-order them as follows:

$$\underbrace{x_1, x_2, \ldots}_{V_0} \quad \underbrace{x_\alpha, x_{\alpha+1}, \ldots}_{W_0} \quad \underbrace{x_\beta, x_{\beta+1}, \ldots}_{V_{-1}} \quad \underbrace{x_\gamma, x_{\gamma+1}, \ldots}_{W_{-1}} \quad \underbrace{x_\delta, x_{\delta+1}, \ldots}_{V_{-\omega}} \quad \underbrace{x_\epsilon, x_{\epsilon+1}, \ldots}_{W_{-\omega}}$$

Then every vertex of $\mathfrak{D}$ is in some $V_{-\zeta}$ or some $W_{-\zeta}$ with $\zeta < \lambda$. Let κ be the lowest ordinal for which $G_{-\kappa} = 0$. Then every vertex of G is ultimately used up in some $V_{-\zeta}$ or $W_{-\zeta}$, $\zeta < \kappa$. We have then the following theorem, in which we let $V = \bigcup_{0 \leqq \alpha < \kappa} V_{-\alpha}$:

THEOREM 2. *If V_0 is a solution of the subsystem $(\mathfrak{D}_0, \succ)$ of the system $(\mathfrak{D}, \succ)$, and if the graph $\mathrm{Cl}(G - G_0)$ is progressively finite, and every vertex of $G - G_0$ is in the predecessor-set $P(\mathfrak{D}_0, G - G_0)$, then V is a maximally satisfactory set.*

THEOREM 3. *If, in addition to the hypotheses of Theorem 2, there exist inverse bases $V_{-\alpha}$ for each α with $1 \leqq \alpha < \kappa$ such that $D^{-1}(V_0, G - G_0) \subset D(V, G)$ and $D^{-1}(V_{-\alpha}, G_{-\alpha}) \subset D(V, G)$, then V is a solution of G and an extension of V_0.*

THEOREM 4. *If, in addition to the hypotheses of Theorem 2, $\succ$ is symmetric,[3] then V is a solution of G and an extension of V_0.*

THEOREM 5. *If the hypotheses of Theorem 1 are satisfied then so are the hypotheses of Theorem 3 above.*

Examples can be constructed easily to show that Theorem 3 above is less restrictive than Theorem 1.

4. If H is a subgraph of G, let $K(x, H) = D(x, H) \smile D^{-1}(x, H)$, $x \in \mathfrak{D}$; let $K(X, H) = \bigcup_{x \in X} K(x, H)$, $X \subset \mathfrak{D}$; let $K^n(X, H) = K(K^{n-1}(X, H), H)$ for $n > 1$. That is, $K^n(X, H)$ denotes the set of vertices of H connected to vertices of X by unoriented one-dimensional chains of length n.

LEMMA 9. *If $\mathfrak{D} - \mathfrak{D}_0 \subset P(V_0, G - G_0)$, then every inverse basic set B of G_{-i-1} has a sink in $K^2(V_{-i}, G_{-i-1})$, $i \geqq 0$.*

A subgraph H of G is termed *progressively bounded at the vertex y* if all progressions of H beginning with y have lengths forming a bounded set of natural numbers. H is termed *progressively bounded* if it is progressively bounded at each of its vertices.

LEMMA 10. *If $\mathfrak{D} - \mathfrak{D}_0 \subset P(V_0, G - G_0)$ and if $\mathrm{Cl}(G - G_0)$ is progressively bounded then every vertex y of $\mathfrak{D} - \mathfrak{D}_0$ is an element of V_{-i} or W_{-i} for some finite ordinal i.*

By a *relative cycle* (of $\mathrm{Cl}(G - G_0)$ modulo V_0 with modulo 2 coefficients) shall be meant an unoriented one-dimensional chain lying in $\mathrm{Cl}(G - G_0)$ except for its set of boundary vertices (possibly empty; that is, absolute cycles are included among the relative cycles) which lies in V_0.

THEOREM 6. *If V_0 is a solution of G_0 such that (1) $\mathrm{Cl}(G - G_0)$ is progressively bounded; (2) there exist no undominated vertices of $\mathfrak{D} - \mathfrak{D}_0$ in any $K^{2n-1}(V_0, G - G_0)$; (3) $\mathrm{Cl}(G - G_0)$ contains no relative cycles of odd length; (4) $\mathfrak{D} - \mathfrak{D}_0 \subset P(V_0, G - G_0)$; then there exists a solution V of G which is an extension of the solution V_0 of G_0.*

THEOREM 7. *Let V be any maximally satisfactory set containing V_0 such that (1) every $v \in V$ belongs to $K^{2n}(V_0, G - G_0)$ for some n; (2) every element of $K^{2m-1}(V_0, G - G_0)$, for each natural number m, is dominated by some element of $\mathfrak{D} - \mathfrak{D}_0$; (3) $\mathrm{Cl}(G - G_0)$ contains no relative cycles of odd length. Then V is a solution of G.*

It is easy to show that the hypotheses of Theorem 7 imply that $V = \bigcup K^{2n}(V_0, G - G_0)$ and $\mathfrak{D} - V = \bigcup K^{2m-1}(V_0, G - G_0)$. Thus Theorem 7 resembles Theorem 1, except that now the parity restrictions are on the unoriented chains rather than oriented ones and we do not restrict the sets $K^n(W_{00}, G - G_0)$. Examples can be constructed easily which are covered by Theorem 7 but not by Theorem 1.

Let $\mu^h(X, G - G_0)$ denote the set of vertices of $G - G_0$ connected to X by an unoriented chain of minimal length h. Then $\mu^h(X, G - G_0) \frown \mu^k(X, G - G_0) = 0$ for $h \neq k$. By $\mu^0(X, G - G_0)$ is meant X.

THEOREM 8. *Let V_0 be a solution of G_0 where G_0 is a conjunct subgraph of G. Let $W_0 = \mathfrak{D}_0 - V_0$ and suppose that every vertex of $\mathfrak{D} - \mathfrak{D}_0$ is connected to $\mathfrak{D}_0$ by some unoriented chain. Let*

$$V = \bigcup_{n=0}^{\infty} \mu^{2n}(V_0, G - G_0) \smile \bigcup_{m=1}^{\infty} \mu^{2m-1}(W_0, G - G_0),$$

$$W = \bigcup_{n=1}^{\infty} \mu^{2n-1}(V_0, G - G_0) \smile \bigcup_{m=0}^{\infty} \mu^{2m}(W_0, G - G_0).$$

Suppose that (1) every element of W is dominated by some element of V; (2) $\mu^h(V_0, G - G_0) \frown \mu^k(W_0, G - G_0) = 0$ if h and k have the same parity; (3) no two elements of the same $\mu^{2n-1}(W_0, G - G_0)$ are adjacent; (4) no two elements of the same $\mu^{2n}(V_0, G - G_0)$ are adjacent. Then V is a solution of G which is an extension of V_0.

THEOREM 9. *Let V_0 be a solution of a conjunct subgraph G_0 of G such that every vertex of $\mathfrak{D} - \mathfrak{D}_0$ is connected to V_0 by some unoriented chain. Let (1) no two elements of the same $\mu^{2i}(V_0, G - G_0)$, $i > 0$, be adjacent; (2) $x \in \mu^{2i-1}(V_0, G - G_0)$ imply that there exists a $j \geqq 0$ such that $x \prec y$ for some $y \in \mu^{2j}(V_0, G - G_0)$. Then $V = \bigcup_{i=0}^{\infty} \mu^{2i}(V_0, G - G_0)$ is a solution of G which is an extension of V_0.*

The conditions of Theorem 9 do not prohibit entirely the existence in $\mathrm{Cl}(G - G_0)$ of adjacent vertices of W, of odd cycles mod 2, or of transitive triples.

Theorems 6–9 may be regarded as variants of Theorem 1.

5. Let G_1 be a conjunct subgraph of G. If x is a vertex of G, let $D(x, G - G_1)$ denote the set of all vertices y of $G - G_1$ such that $x \succ y$. If X is a set of vertices of G, let $D(X, G - G_1) = \bigcup_{x \in X} D(x, G - G_1)$. For $n > 1$, let $D^n(X, G - G_1) = D(D^{n-1}(X, G - G_1), G - G_1)$. By the *successor-set* of X *relative to* $G - G_1$ is meant the set $S(X, G - G_1) = \bigcup_{n=1}^{\infty} D^n(X, G - G_1)$.

THEOREM 10. *Let G_1 be a conjunct subgraph of G. Let V_1 be a solution of G_1, and $W_1 = \mathfrak{D}_1 - V_1$ where $\mathfrak{D}_1$ is the set of vertices of G_1. Suppose that: (1) $D(V_1, G) \frown D^{2n}(V_1, G - G_1) = D(V_1, G) \frown D^{2n-1}(W_1, G - G_1) = 0$ and $V_1 \frown D^{2n+1}(V_1, G - G_1) = V_1 \frown D^{2n}(W_1, G - G_1) = 0$ for every positive integer n; (2) if $h > 0$ and $k > 0$ are of the same parity then $D^h(V_1, G - G_1) \frown D^k(W_1, G - G_1) = 0$; if $h > 0$ and $k > 0$ are of different parities then $D^h(V_1, G - G_1) \frown D^k(V_1, G - G_1) = D^h(W_1, G - G_1) \frown D^k(W_1, G - G_1) = 0$; (3) every vertex of $G - G_1$ is in $S(\mathfrak{D}_1, G - G_1)$. Then solutions of G which are extensions of V_1 exist.*

COROLLARY. *Let G_1 be a conjunct subgraph of G. Let V_1 be a solution of G_1. Suppose that: (a) no vertex of any $S(x_1, G - G_1)$, $x_1 \in \mathfrak{D}_1$, is adjacent to any vertex of G_1 other than x_1; and if x_1 and x_1' are any two distinct vertices of G_1 then $S(x_1, G - G_1) \frown S(x_1', G - G_1) = 0$. (b) no $S(x_1, G - G_1) \smile (x_1)$, $x_1 \in \mathfrak{D}_1$, contains an odd cycle modulo 2; (c) every vertex of $G - G_1$ is in $S(\mathfrak{D}_1, G - G_1)$. Then solutions of G which are extensions of V_1 exist.*

Let $\mathfrak{D}_i$ be the vertex-set of G_i. If G_0 intersects every component of G, then $\mathfrak{D} = \bigcup_{i=0}^{\infty} \mathfrak{D}_i$. For then every vertex of G is joined to some vertex of G_0 by a finite unoriented chain and therefore lies in some G_i. In particular, this is true if G is connected.

Now suppose G_0 is a non-empty conjunct subgraph of G, and let V_0 be a solution of G_0. For each $n = 1, 2, 3, \ldots$, let G_{2n-1} be constructed by adjoining to G_{2n-2} the vertices of $P(\mathfrak{D}_{2n-2}, G - G_{2n-2})$ and all the arcs joining all these vertices; and let G_{2n} be constructed by adjoining to G_{2n-1} the vertices of $S(\mathfrak{D}_{2n-1}, G - G_{2n-1})$ and all the arcs joining all these vertices.

Then each G_i is a conjunct subgraph of G_{i+1}; for, $x, y \in G_i$, $x \succ y$ relative to G_{i+1} implies $x \succ y$ relative to G_i since at least one end-point of every arc in $G_{i+1} - G_i$ is not in G_i.

THEOREM 11. *Let G_0 be a conjunct subgraph of G, and let V_0 be a solution of G_0. Suppose G_0 intersects every component of G. If, for every even i, G_i satisfies conditions (1), (2), (3) of Theorem 1 relative to G_{i+1}, and, for every odd i, G_i satisfies conditions (1), (2) of Theorem 10 relative to G_{i+1}, then solutions of G which are extensions of V_0 exist.*

Theorem 10 is an analogue of Theorem 1 using successors instead of predecessors. Similar analogues of other theorems can be proved, and alternating procedures like that of Theorem 11 can be applied.

[1] To appear under the title "Solutions of Irreflexive Relations" in the *Annals of Mathematics*. This paper will be referred to as SOIR.

[2] This is a slight modification of the notation of SOIR.

[3] As to Theorem 4, the fact that if $\succ$ is symmetric then every maximally satisfactory set is a solution is established in von Neumann, J., and Morgenstern, O., *Theory of Games and Economic Behavior*, Princeton, 1944, 2nd ed., 1947.

A FIRST APPROXIMATION TO HOMOTOPY THEORY

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1. The suspension map, originally introduced by Freudenthal for the study of the homotopy groups of spheres, has proved important in general homotopy questions, and it has been found that within the "suspension range"[1] the situation is simpler than in the general case. In this note we show how, by passing to a direct limit of homotopy classes under suspension, it is possible to obtain a new category which is simpler in structure than the homotopy category (of topological spaces and homotopy classes of maps). The new category, called the S-category, has the important property that, for it, suspension is always an isomorphism. The homotopy classes of maps of a suspension, modulo the poles, into a space with a base point can be made into a group by an addition called *track addition*.[2] It follows that in the S-category the mappings from one given object to an-

other always form a group and form a ring when the two objects are the same.

The S-category, because of these special properties, appears to be a natural first approximation to the study of homotopy properties. There is a natural map of the homotopy category into the S-category, and we show in §5 that within the suspension range this map is an isomorphism, at least when the spaces are CW-complexes. Hence, all results about the S-category can be applied to the homotopy category within this limit.

It is clearly desirable to include relative homotopy in the S-category. We do this comprehensively by means of "carriers," which are defined in §2. The third section is concerned with suspension and a certain exact sequence. In §4 we set up the new category and establish some of its properties.

Details of the proofs are omitted and will appear in a subsequent paper.

2. We shall be concerned with triples $(X, x_*; \alpha)$, where X is a space, $x_* \in X$ is a base point and α is a collection of subsets of X such that $x_* \in A$, for each $A \in \alpha$, and the set, $\{x_*\}$, consisting of the single point x_*, belongs to α.

A *carrier*, ϕ, from $(X, x_*; \alpha)$ to $(Y, y_*; \beta)$ is an order preserving map of α into β (i.e., $\phi A \subset \phi A'$ if $A \subset A'$, $A, A' \in \alpha$) such that $\phi\{x_*\} = \{y_*\}$. For each $(X, x_*; \alpha)$ there is an identity carrier, and if ϕ is a carrier from $(X, x_*; \alpha)$ to $(Y, y_*; \beta)$ and ψ is a carrier from $(Y, y_*; \beta)$ to $(Z, z_*; \gamma)$, their composition, $\psi\phi$, is a carrier from $(X, x_*; \alpha)$ to $(Z, z_*; \gamma)$. If ϕ_1, ϕ_2 are two carriers from $(X, x_*; \alpha)$ to $(Y, y_*; \beta)$, we write $\phi_1 \leq \phi_2$ if, and only if, $\phi_1 A \subset \phi_2 A$ for each $A \in \alpha$.

A continuous mapping $f: X \to Y$ is called *ϕ-admissible*, or a *ϕ-map*, if, and only if, $fA \subset \phi A$ for each $A \in \alpha$. Clearly the composition of a ϕ-map with a ψ-map is a $\psi\phi$-map. Similarly a homotopy $f_t: X \to Y$ is called a *ϕ-homotopy* if $f_t A \subset \phi A$ for each $t \in I$ and $A \in \alpha$. Obviously ϕ-homotopy is an equivalence relation between ϕ-maps, and the corresponding equivalence classes will be called *ϕ-homotopy classes*. From the above remarks about the composition it is clear that there is a category[3] $\mathfrak{C}$, whose objects are the triples $(X, x_*; \alpha)$ and whose mappings are pairs $(\hat{f}, \phi)$ where ϕ is a carrier from $(X, x_*; \alpha)$ to $(Y, y_*; \beta)$ and $\hat{f}$ is a ϕ-homotopy class of maps $X \to Y$. It is clear that no generality is lost by assuming that, for each $(X, x_*; \alpha)$, $X \in \alpha$.

If ϕ is a carrier, we let $\pi(\phi)$ denote the collection of ϕ-homotopy classes. Since the constant map $X \to y_*$ is ϕ-admissible it determines a distinguished element in $\pi(\phi)$. If $\phi_1 \leq \phi_2$, there is a natural map $\pi(\phi_1) \to \pi(\phi_2)$ because each ϕ_1-map is a ϕ_2-map.

3. Let E^1 denote the interval $-1 \leq t \leq 1$. By the *cone* CX we mean the space obtained from $X \times E^1$ by shrinking $(X \times -1) \cup (x_* \times E^1)$ to a point. The *suspension* SX is defined to be the space obtained from

$X \times E^1$ by shrinking $(X \times -1) \cup (x_* \times E^1) \cup (X \times 1)$ to a point. We use (x, t) to denote the point of CX or SX corresponding to the point $(x, t) \in X \times E^1$. We shall denote $(x, 1) \in CX$ by x and regard X as thus imbedded[4] in CX. We also write $x_* = (x_*, t) \in SX$ and use x_* as base point for CX and SX. Clearly if $A \subset X$ then $CA \subset CX$ and $SA \subset SX$.

If α is a collection of subsets of X, we define $C\alpha$ to be the union of α with the set of cones CA, for each $A \in \alpha$. We also define $S\alpha$ as the set of sets SA $(A \in \alpha)$. We define $T(X, x_*; \alpha) = (TX, x_*; T\alpha)$ for $T = C$ or S. If ϕ is a carrier from $(X, x_*; \alpha)$ to $(Y, y_*; \beta)$, we define $T\phi$ to be the obvious carrier from $T(X, x_*; \alpha)$ to $T(Y, y_*; \beta)$. If $f: X \to Y$, we define $Tf: TX \to TY$ by $(Tf)(x, t) = (fx, t)$. If f is ϕ-admissible, Tf is $T\phi$-admissible. Hence, we have two functors, C and S, from $\mathfrak{C}$ to $\mathfrak{C}$. There is a natural equivalence between CS and SC by means of which we identify CS with SC. We define inductively $S^{n+1} = SS^n$.

Let ϕ be a carrier from $(X, x_*; \alpha)$ to $(Y, y_*; \beta)$. We define a carrier ϕ^n_m, from $S^m(X, x_*; \alpha)$ to $S^n(Y, y_*; \beta)$, by $\phi^n_m S^m A = S^n \phi A$ for $A \in \alpha (m, n \geq 0)$. If $\phi \leq \psi$ are carriers from $(X, x_*; \alpha)$ to $(Y, y_*; \beta)$, we define a carrier, $(\psi, \phi)_1$, from $C(X, x_*; \alpha)$ to $(Y, y_*; \beta)$ by $(\psi, \phi)_1 A = \phi A$, $(\psi, \phi)_1 CA = \psi A$. Under the identification of the functors $S^m C$ and CS we see that $(\psi^n_m, \phi^n_m)_1 = ((\psi, \phi)_1)^n_m$ and we define $(\psi, \phi)^n_{m+1}$ to be either of these.

Since $\phi^n_m \leq \psi^n_m$, there is a natural map $\pi(\phi^n_m) \to \pi(\psi^n_m)$. Since $S^m X$ is obtained from $CS^{m-1}X$ by collapsing $S^{m-1}X$ to x_* $(m \geq 1)$, we obtain a mapping $\pi(\psi^n_m) \to \pi(\psi, \phi)^n_m$ by composing each ψ^n_m-map $S^m X \to S^n Y$ with the collapsing map $CS^{m-1}X \to S^m X$. If $f: CS^{m-1}X \to S^n Y$ is $(\psi, \phi)^n_m$-admissible, then $f|S^{m-1}X$ is ϕ^n_{m-1}-admissible. Therefore we obtain, by the restriction of a map of $CS^{m-1}X$ to $S^{m-1}X$, a mapping $\pi(\psi, \phi)^n_m \to \pi(\phi^n_{m-1})$. These mappings fit together into the sequence[5]

$$(3.1)_n \quad \cdots \to \pi(\phi^n_m) \to \pi(\psi^n_m) \to \pi(\psi, \phi)^n_m \to \pi(\phi^n_{m-1}) \to \cdots \to \pi(\psi^n_0).$$

THEOREM 3.2. *For each $n \geq 0$ the sequence $(3.1)_n$ is exact.*

Exactness means that the inverse image of the distinguished element at any stage is the image of the preceding map.

The suspension functor S maps $\pi(\phi^n_m)$ to $\pi(\phi^{n+1}_{m+1})$, for each carrier ϕ and commutes with the maps of the exact sequence $(3.1)_n$. Therefore it maps the sequence $(3.1)_n$ into the sequence $(3.1)_{n+1}$ by sending each term into the corresponding term with both indices increased by 1.

If $m \geq 1$ the sets $\pi(\psi, \phi)^n_{m+1}$, $\pi(\phi^n_m)$, $\pi(\psi^n_m)$ are groups with the track addition. If $m \geq 2$ these groups are abelian.[6] The group structure is compatible with the maps of the exact sequence $(3.1)_n$ and the suspension map. Hence, each sequence $(3.1)_n$ consists of groups and homomorphisms except for the last three terms.

4. Let ϕ mean the same as before and consider the sequence of suspension maps

$$\sum: \pi(\phi) \to \cdots \to \pi(S^k\phi) \to \pi(S^{k+1}\phi) \to \cdots.$$

Let $\pi_S(\phi) = \varinjlim \Sigma$. The elements of $\pi_S(\phi)$ will be called *S-ϕ-mappings*. The S-ϕ-mappings, for every carrier ϕ, evidently constitute a category $\mathfrak{C}_S$, the *S*-category, in which the objects are the triples $(X, x_*; \alpha)$ and multiplication is defined by composition. Since $\pi(S^k\phi)$ is an abelian group if $k \geq 2$, so is $\pi_S(\phi)$. A functor $\mathfrak{C} \to \mathfrak{C}_S$ is defined in the obvious way.

For every (positive and negative) integer m we define

$$\pi_m(\phi) = \begin{cases} \pi_S(\phi_m^0) & \text{if } m \geq 0 \\ \pi_S(\phi_0^{-m}) & \text{if } m < 0. \end{cases}$$

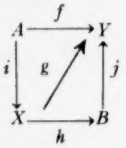
If $\phi \leq \psi$ we define $\pi_m(\psi, \phi) = \pi_{m-1}((\psi, \phi)_1)$, where $(\psi, \phi)_1$ means the same as in §3. By passing to the direct limit of the sequences $(3.1)_n$, with respect to the suspension maps, we obtain the following exact sequence of homomomorphisms of abelian groups

$$(4.1) \qquad \cdots \to \pi_m(\phi) \to \pi_m(\psi) \to \pi_m(\psi, \phi) \to \pi_{m-1}(\phi) \to \cdots,$$

which extends to infinity in both directions.

If ϕ' is a carrier from $(Y, y_*; \beta)$ to $(Z, z_*; \gamma)$ there is induced by composition a pairing of $\pi_S(\phi)$ and $\pi_S(\phi')$ to $\pi_S(\phi'\phi)$. In particular, if $\iota: \alpha \to \alpha$, $\kappa: \beta \to \beta$ are the identical carriers, then $\pi_S(\iota)$, $\pi_S(\kappa)$ are rings, which operate on $\pi_S(\phi)$ from the right and left respectively.

Let $A \subset X$, $B \subset Y$ be such that $x_* \in A$, $y_* \in B$. Let $i: A \to X$ and $j: B \to Y$ denote the inclusion maps. Consider the extension and deformation problems indicated in the diagram



In the "homotopy" extension problem f is given and we ask if there exists a g such that $f \simeq gi$. In the deformation problem g is given and we seek an h such that $g \simeq jh$. Replacing the maps by the corresponding *S*-mappings we describe[7] $\{f\}$ as *S-extendible* if $\{f\} = \{g\}\{i\}$ for some *S*-mapping $\{g\}: (X, x_*) \to (Y, y_*)$. Similarly $\{g\}$ is *S-deformable* if $\{g\} = \{j\}\{h\}$ for some *S*-mapping $\{h\}: (X, x_*) \to (B, y_*)$.

For the deformation problem let $\alpha = \{X, \{x_*\}\}$, $\beta = \{Y, B, \{y_*\}\}$ and define $\phi\{x_*\} = \psi\{x_*\} = \{y_*\}$, $\phi X = B$, $\psi X = Y$. Then $\phi \leq \psi$ and $\{g\} \in \pi_0(\psi)$. Consider the following portion of the exact sequence (4.1)

$$\pi_0(\phi) \to \pi_0(\psi) \to \pi_0(\psi, \phi).$$

To say that $\{g\}$ is *S*-deformable is to say that it is in the image of $\pi_0(\phi)$;

or, by exactness, that it maps into zero in $\pi_0(\psi, \phi)$. Hence, the image of $\{g\}$ in $\pi_0(\psi, \phi)$ is the obstruction to the S-deformability of $\{g\}$.

For the extension problem let $\alpha = \{X, A, \{x_*\}\}$ and $\beta = \{Y, \{y_*\}\}$. We define $\phi\{x_*\} = \psi\{x_*\} = \{y_*\}$, $\phi X = \psi X = Y$, $\phi A = \{y_*\}$, $\psi A = Y$. Then $\phi \leq \psi$. We define also a carrier χ, from A, $\{x_*\}$ to Y, $\{y_*\}$, by $\chi A = Y$, $\chi\{x_*\} = \{y_*\}$. Then the $(\psi, \phi)^{n+1}_{n+1}$-homotopy classes of maps $CS^nX \to S^{n+1}Y$ are isomorphic[8] to the χ^{n+1}_{n+1}-homotopy classes of maps $S^{n+1}A \to S^{n+1}Y$. Hence $\pi_0(\psi, \phi) \approx \pi_0(\chi)$ and from (4.1) we have the following exact sequence

$$\pi_0(\psi) \to \pi_0(\chi) \to \pi_{-1}(\phi).$$

To say that $\{f\} \in \pi_0(\chi)$ is S-extendible is to say that it is in the image of $\pi_0(\psi)$. Hence the image of $\{f\}$ in $\pi_{-1}(\phi)$ is the obstruction to the S-extendibility of $\{f\}$.

5. Let X be a connected CW complex having exactly one 0-cell[9] x_* and let α be the collection of subcomplexes of X. By a *closed q-cell* of X we shall mean the smallest subcomplex which contains a given q-cell of X. We shall denote a closed q-cell by σ^q. For each $q \geq 0$ let n_q be the smallest integer such that $q \leq 2n_q - 1$. Let $(Y, y_*; \beta)$ be a triple such that, for each $B \in \beta$, there is a homotopy f_t: $B \to B$, in which $f_0 = 1$, $f_tU \subset U$, $f_1U = y_*$, where U is some relatively open neighborhood of y_* in B. Let ϕ be a carrier from $(X, x_*; \alpha)$ to $(Y, y_*; \beta)$.

THEOREM 5.1. *If $\phi\sigma^q$ is $(n_q - 1)$-connected for each closed q-cell $\sigma^q \in \alpha$ and each $q \geq 0$, then S maps $\pi(\phi)$ onto $\pi(S\phi)$. If each $\phi\sigma^q$ is $(n_{q+1} - 1)$-connected, then S maps $\pi(\phi)$ onto $\pi(S\phi)$ in 1-1 fashion.*

If the hypotheses of the theorem are satisfied for X, Y and ϕ, then they are also satisfied for SX, SY, and $S\phi$. Hence, under the hypotheses of the second half of (5.1), the natural map $\pi(\phi) \to \pi_S(\phi)$ is a 1-1 correspondence. Thus $\pi(\phi)$ can be given a group structure by means of this correspondence.

Let Y and B be $(q - 1)$-connected, let A be a subcomplex of X and let dim $X \leq 2q - 2$. Then the extension and deformation problems are equivalent to the S-extension and S-deformation problems.

* John Simon Guggenheim Fellow 1952–3.

[1] In the case of mappings of a space X into an $(n - 1)$-connected space Y the suspension range for dim X is dim $X \leq 2n - 2$.

[2] Cf. a paper by Barratt, M. G., to be published in *Proc. London Math. Soc.*

[3] For the concepts of category and functor see Eilenberg, Samuel, and MacLane, Saunders, General Theory of Natural Equivalences, *Trans. Am. Math. Soc.*, **58**, 231–294 (1945).

[4] This is an "abuse of language" because (x, t) may already be a point of X.

[5] This is a generalization of an exact sequence due to M. G. Barratt; see reference 2.

[6] See reference 2 for details concerning the track addition.

[7] $\{f\}$ denotes the S-mapping determined by f. An S-mapping $(X, x_*) \to (Y, y_*)$, means an S-ϕ-mapping, where $\alpha = \{X, \{x_*\}\}$, $\beta = \{Y, \{y_*\}\}$, $\phi X = Y$, $\phi\{x_*\} = \{y_*\}$.

[8] We need here the assumption that CS^nA is a (strong) deformation retract of CS^nX for some $n \geq 0$ and hence for all sufficiently large values of n. This is so if X is a CW-complex and A is a subcomplex.

[9] Any connected CW complex can be transformed, without altering its homotopy type, into one which has but a single o-cell by pinching a suitable tree to a point.

COHOMOLOGY AND THE RIEMANN-ROCH THEOREM

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1. Let M be a complex-analytic manifold of (complex) dimension n, and let S be a non-singular analytic subvariety of M of dimension $n - 1$. At each point $p \in S$ we can introduce local analytic coordinates $z_p^1, \ldots, z_p^n$ on M with center at p such that $z_p^1 = 0$ on S and such that $z_p^2, \ldots, z_p^n$ become local coordinates on S. We denote by $\Omega^r = \Omega^r(M)$ the faisceau of the germs of holomorphic r-forms, and by Ω_S^r the faisceau of the germs of meromorphic r-forms which are multiples of $-S$. If $\tau = \tau_p$ is the germ of a meromorphic r-form defined in a neighborhood U_p of a point $p \in S$, we say that τ is a multiple of $-S$ if $z_p^1\tau$ is holomorphic throughout U_p.

Let $p, q \in S$; if $U_p \cap U_q \neq 0$ we define $f_{pq} = (z_p^1/z_q^1)_S$ in $U_p \cap U_q$ where $(z_p^1/z_q^1)_S = z_p^1/z_q^1 \mid S$ is the restriction of the function z_p^1/z_q^1 to S. The system $\{f_{pq}\}$ defines a principal fiber space over S with fiber C^* (the multiplicative group of complex numbers). If there exists a divisor D on S defined by a system $\{h_p\}$ of functions h_p where h_p is meromorphic in a neighborhood U_p on S of the point p and where $h_p/h_q = f_{pq}$ in $U_p \cap U_q$, we say that D is a characteristic divisor of S. We suppose in the following that a characteristic divisor D exists (it always exists if M is an algebraic variety).

Let $p \in S$, and let

$$\varphi = \sum_{i_1 < \ldots < i_s} \varphi_{i_1 \ldots i_s}\, dz^{i_1} \ldots dz^{i_s}$$

be an arbitrary form of degree s defined throughout a neighborhood U_p of the point $p \in S$ where $dz^i = dz^\alpha$ if $1 \leq i = \alpha \leq n$, $dz^i = d\bar{z}^\alpha$ if $n + 1 \leq i = \alpha + n \leq 2n$. We define

$$\nu_p\varphi = \sum_{1 < i_2 < \ldots < i_s} \varphi_{1 i_2 \ldots i_s}\, dz^{i_2} \ldots dz^{i_s}$$

if $s \geq 1$, $\nu_p\varphi = 0$ if $s = 0$. With this convention, the Poincaré residue $\mathcal{P}(\tau)$ of the germ τ_p of a meromorphic r-form is $(\nu_p \cdot z_p^1\tau_p)_S$, while its Severi residue $\mathcal{Q}(\tau)$ is equal to $(z_p^1\tau_p/h_p)_S$. Let τ_p be regarded as a current in U_p of type $(r, 0)$, and let $\bar{\partial}$ be the operator of exterior differentiation which

maps currents of type (r, s) into currents of type $(r, s+1)$. If ψ is an arbitrary form of type $(n-r, n-1)$ and of class C^∞ with carrier contained in a compact subdomain of U_p, a simple calculation shows that

$$\bar{\delta}\tau_p[\psi] = 2\pi i \cdot \mathfrak{R}_p[\psi] \tag{1.1}$$

where

$$\begin{aligned}\mathfrak{R}_p[\psi] &= \int_S \{\nu_p(z_p^1\tau_p)\psi + (-1)^r(z_p^1\tau_p)\nu_p\psi\} \\ &= \int_S \{\mathcal{P}(\tau_p)\psi + (-1)^r\mathcal{Q}(\tau_p)h_p\nu_p\psi\}.\end{aligned}$$

We call $\mathfrak{R}_p = \mathfrak{R}(\tau_p)$ the residue of τ_p, and we denote by $\mathfrak{R}_S^r$ the faisceau of the germs of all these residues. We have the canonical isomorphism

$$\mathfrak{R}_S^r \approx \Omega^{r-1}(S) + \Omega_D^r(S) \tag{1.2}$$

Next, the sequence of faisceaux

$$0 \to \Omega^r \xrightarrow{i} \Omega_S^r \xrightarrow{\delta} \mathfrak{R}_S^r \to 0 \tag{1.3}$$

is exact (i denotes the inclusion map). In fact, let $\omega_1 \in (\Omega^{r-1}(S))_p$, $\omega_2 \in (\Omega_D^r(S))_p$, $p \in S$, and define

$$\tau_p = d\log z_p^1 \cdot \omega_1 + \omega_2 h_p/z_p \tag{1.4}$$

Then $\tau_p \in (\Omega_S^r)_p$, and $\mathcal{P}(\tau_p) = \omega_1$, $\mathcal{Q}(\tau_p) = \omega_2$; hence the mapping $\bar{\delta}: \Omega_S^r \to \mathfrak{R}_S^r$ is onto.

The exact cohomology sequence corresponding to (1.3) is

$$\ldots \to H^{s-1}(M, \mathfrak{R}_S^r) \xrightarrow{\delta^*} H^s(M, \Omega^r) \xrightarrow{i^*} H^s(M, \Omega_S^r) \xrightarrow{\bar{\delta}^*} H^s(M, \mathfrak{R}_S^r) \xrightarrow{\delta^*} H^{s+1}(M, \Omega^r) \to \ldots$$

Hence

$$\dim H^s(M, \Omega_S^r) = \dim\{i^*H^s(M, \Omega^r)\} + \dim\{\bar{\delta}^* H^s(M, \Omega_S^r)\}, \tag{1.5}$$

that is

$$\begin{aligned}\dim H^s(M, \Omega_S^r) = {} & \dim\{H^s(M, \Omega^r)/\delta^* H^{s-1}(M, \mathfrak{R}_S^r)\} + \dim H^s(M, \mathfrak{R}_S^r) - \\ & \dim H^{s+1}(M, \Omega^r) + \dim\{H^{s+1}(M, \Omega^r)/\delta^* H^s(M, \mathfrak{R}_S^r)\}.\end{aligned} \tag{1.6}$$

Let $A^{r,s}$ be the faisceau of germs of currents of type (r, s), $F^{r,s}$ the subfaisceau of germs of currents closed under $\bar{\delta}$. We have the exact sequence[1]

$$0 \to F^{r,s} \xrightarrow{i} A^{r,s} \xrightarrow{\bar{\delta}} F^{r,s+1} \to 0,$$

and from the corresponding exact cohomology sequence we obtain the canonical isomorphism (cf. the note[1])

$$H^s(M, \Omega^r) \approx H^{r,s}(M)$$

or, more generally,

$$H^t(M, F^{r,s}) \approx H^{r,s+t}(M) \tag{1.7}$$

where

$$H^{r,s}(M) = H^0(M, F^{r,s})/\bar{\partial} H^0(M, A^{r,s-1}) \tag{1.8}$$

is the $\bar{\partial}$-cohomology of currents of type (r, s). If M is a compact Kähler manifold, $H^{r,s}(M)$ is isomorphic to the vector space of harmonic forms on M of type (r, s).

Next, on the basis of (1.3),

$$H^s(M, \mathfrak{R}_S^r) \approx H^s(S, \Omega^{r-1}) + H^s(S, \Omega_D^r). \tag{1.9}$$

Substituting from (1.7) and (1.9) into (1.6), we obtain the dimension formula (Riemann-Roch theorem)

$$\dim H^s(M, \Omega_S^r) = \dim H^{r-1,s}(S) + \dim H^s(S, \Omega_D^r) - \dim H^{r,s+1}(M) + \dim \{H^{r,s}(M)/\delta^* H^{s-1}(M, \mathfrak{R}_S^r)\} + \dim \{H^{r,s+1}(M)/\delta^* H^s(M, \mathfrak{R}_S^r)\}. \tag{1.10}$$

Let

$$a^r(M) = \sum_{s=0}^{n} (-1)^s \dim H^s(M, \Omega^r),$$

$$a_S^r(M) = \sum_{s=0}^{n} (-1)^s \dim H^s(M, \Omega_S^r).$$

If we multiply both sides of (1.10) by $(-1)^s$ and sum on s, we obtain

$$a_S^r(M) = a^{r-1}(S) + a_D^r(S) + a^r(M). \tag{1.11}$$

2. Let $\mathfrak{U} = \{U_i\}$ be a locally finite open covering of M, and let $N(\mathfrak{U})$ be its nerve. To each simplex $u = (U_{i_0}, \ldots, U_{i_s}) \in N(\mathfrak{U})$ we associate the open set $X(u) = U_{i_0} \cap \ldots \cap U_{i_s}$ where $X(u) \neq 0$. Given a faisceau F, a cochain f is a function of simplex u such that $f(u) \in \Gamma(F, X(u))$ where $\Gamma(F, X(u))$ is the module of sections of F over $X(u)$. The support of f is defined to be the union of the supports of the values $f(u)$ for all $u \in N(\mathfrak{U})$. The cochains then form a graded module with coboundary which we denote by $C(\mathfrak{U}, F)$. Let $Z(\mathfrak{U}, F)$ be the submodule of cocycles, $B(\mathfrak{U}, F)$ the submodule of coboundaries, and define $H(\mathfrak{U}, F) = Z(\mathfrak{U}, F)/B(\mathfrak{U}, F)$.

Given two coverings $\mathfrak{U} = \{U_i\}$, $\mathfrak{V} = \{V_j\}$ of M, we say that $\mathfrak{V}$ is subordinate to $\mathfrak{U}$, $\mathfrak{V} \prec \mathfrak{U}$, if each V_j is contained in some U_i. Then $H^s(M, F)$ is defined to be the limit module of the $H^s(\mathfrak{U}, F)$ over all locally finite open coverings of M. Let $\pi(\mathfrak{U}): H(\mathfrak{U}, F) \to H(M, F)$ be the natural map. Given $h \in H(M, F)$, there exists a covering $\mathfrak{U}$ and a $g \in H(\mathfrak{U}, F)$ such that $h = \pi(\mathfrak{U}) \cdot g$.

Let $\mathfrak{U} = \mathfrak{U}(M) = \{U_i\}$, $\mathfrak{V} = \mathfrak{V}(S) = \{V_j\}$ be locally finite coverings of M and S, respectively, and let $\mathfrak{U}_S = \{U_j | S \cap U_j \neq \phi\}$ be such that each

$U_j \in \mathfrak{U}_S$ is contained in a domain of local coordinates $z_i^1, \ldots, z_i^n$ where $z_i^1 = 0$ on S. We suppose that $\mathfrak{U}_S = \{U_j | U_j = V_j \times [|z_j| < \epsilon_j], V_j \in \mathfrak{V}(S)\}$, and we define $\kappa \in C^0(\mathfrak{U}, \Omega_S^1)$, $\lambda \in C^0(\mathfrak{U}, \Omega_S^0)$ by the formulae

$$\kappa = \begin{cases} d \log z_i^1 & \text{in } U_i, U_i \in \mathfrak{U}_S \\ 0 & \text{in } U_i, U_i \notin \mathfrak{U}_S, \end{cases}$$

$$\lambda = \begin{cases} h_i/z_i^1 & \text{in } U_i, U_i \in \mathfrak{U}_S \\ 0 & \text{in } U_i, U_i \notin \mathfrak{U}_S. \end{cases}$$

Here $\{h_i\}$ is a system on S defining the characteristic divisor D, $h_i/h_j = (z_i^1/z_j^1)_S$. Given $\omega_1 \in Z^s(\mathfrak{V}(S), \Omega^{r-1})$, $\omega_2 \in Z^s(\mathfrak{V}(S), \Omega_D^r)$, we define (cf. (1.4))

$$\tau = \kappa \circ \omega_1 + \lambda \circ \omega_2. \tag{2.1}$$

If we denote the coboundary operator by $\delta = \delta(\mathfrak{U})$, it is readily verified that $\delta\tau \in Z^{s+1}(\mathfrak{U}, \Omega^r)$. Hence the mapping $\omega_1 + \omega_2 \to \delta\tau$ defines the homormorphism

$$H^s(\mathfrak{U}, \mathfrak{R}_S^r) \to H^{s+1}(\mathfrak{U}, \Omega^r). \tag{2.2}$$

We now describe the homomorphism

$$H^t(\mathfrak{U}, F^{r,s}) \to H^{r,s+t}(M) \tag{2.3}$$

corresponding to (1.7). Given $\omega \in Z^t(\mathfrak{U}, F^{r,s})$, then $\omega \in Z^t(\mathfrak{U}, A^{r,s})$ and, since $A^{r,s}$ is a fine faisceau, we have $H^t(\mathfrak{U}, A^{r,s}) = 0$, $t > 0$. Therefore there exists a λ_0 such that

$$\omega = \delta\lambda_0, \qquad \lambda_0 \in C^{t-1}(\mathfrak{U}, A^{r,s}),$$

and we define

$$\sigma_1 = \bar{\partial}\lambda_0, \qquad \sigma_1 \in Z^{t-1}(\mathfrak{U}, A^{r,s+1}).$$

Since $\sigma_1 \in Z^{t-1}(\mathfrak{U}, A^{r,s+1})$, there exists a λ_1 such that

$$\sigma_1 = \delta\lambda_1, \qquad \lambda_1 \in Z^{t-2}(\mathfrak{U}, A^{r,s+1}).$$

Writing $\omega = \sigma_0$, we obtain the equations

$$\sigma_\nu = \delta\lambda_\nu, \ \sigma_{\nu+1} = \bar{\partial}\lambda_\nu, \ \sigma_\nu \in Z^{t-\nu}(\mathfrak{U}, F^{r,s+\nu}), \ \lambda_\nu \in C^{t-\nu-1}(\mathfrak{U}, A^{r,s+\nu})$$

for $\nu = 0, 1, \ldots, t-1$. The homomorphism (2.3) is then defined by $\omega \to \sigma_t \in \Gamma(F^{r,s+t}, M)$.

In particular, if $\omega = \delta\tau$ where τ is defined by (2.1), we may take $\lambda_0 = \tau$ and we obtain (cf. (1.1))

$$\sigma_1[\psi] = 2\pi i \cdot \int_S \{\omega_1\psi + (-1)^r \omega_2 h_i \nu_i \psi\}. \tag{2.4}$$

We remark that, given $h \in H^s(M, \mathfrak{R}_S^r)$, there exist coverings $\mathfrak{U}(M)$, $\mathfrak{V}(S)$

and cocycles $\omega_1 \in Z^s(\mathfrak{U}(S), \Omega^{r-1})$, $\omega_2 \in Z^s(\mathfrak{U}(S), \Omega^r_D)$ defining an element $g \in H^s(\mathfrak{U}, \mathfrak{R}^r_S)$ such that the $\delta\tau$ constructed above defines an element of $H^{s+1}(\mathfrak{U}, \Omega^r)$ which, in the limit, is the image of h in the Bockstein homomorphism

$$\delta^*: H^s(M, \mathfrak{R}^r_S) \to H^{s+1}(M, \Omega^r).$$

A similar remark applies to (2.3). We may suppose, therefore, that the above constructions correspond to the homomorphisms of the exact cohomology sequences associated with them.

Assume now that M is a compact Kähler manifold. Then $H^{r,\,s+1}(M)$ is isomorphic to the space of harmonic forms on M of the type $(r, s+1)$ and

$$H^{r,\,s+1}(M)/\delta^* H^s(M, \mathfrak{R}^r_S) \approx \{\varphi \mid \varphi \in H^{r,\,s+1}(M), (\sigma_{s+1}, \varphi) = \sigma_{s+1}[*\varphi] = 0\}. \quad (2.5)$$

In particular, taking $s = 0$, $r = 0$, n, we obtain by (2.4)

$$H^{n,\,1}(M)/\delta^* H^0(M, \mathfrak{R}^0_S) \approx \{\varphi \mid \varphi \in H^{n-1,\,0}(M), \varphi_S = 0\},$$
$$H^{0,\,1}(M)/\delta^* H^0(M, \mathfrak{R}^n_S) \approx \{\varphi \mid \varphi \in H^{1,\,0}(M), \varphi_S = 0\}.$$

Therefore, in the case of a compact Kähler manifold, we have by (1.10)

$$\dim H^s(M, \Omega^r_S) = \dim H^{r-1,\,s}(S) + \dim H^s(S, \Omega^r_D) - \dim H^{r,\,s+1}(M) + \dim \{\varphi \mid \varphi \in H^{r,\,s}(M), (\sigma_s, \varphi) = 0\} + \dim \{\varphi \mid \varphi \in H^{r,\,s+1}(M), (\sigma_{s+1}, \varphi) = 0\}. \quad (2.6)$$

If $s = 0$, $r = 0$ or n, (2.6) reduces to formulae which have been obtained by Kodaira.[2, 3]

Finally, if M is compact Kähler, we have, taking $r = 0$, n in (1.11)

$$a^0_S(M) = a^0_D(S) + a^0(M) \quad (2.7)$$

$$a^0(S) = (-1)^{n-1}\{a^n_S(M) - a^n(M)\} \quad (2.8)$$

where in (2.8) we have used the fact that $a^{n-1}(S) = (-1)^{n-1}a^0(S)$. Given an arbitrary divisor D in the compact Kähler manifold M, we therefore define

$$a(D) = a^0(D) = (-1)^{n-1}\{a^n_D(M) - a^n(M)\}. \quad (2.9)$$

3. Let M be a complex-analytic manifold, and let D be an arbitrary divisor on M. We denote by Ω^r_D the faisceau of the germs of meromorphic r-forms which are multiples of $-D$. If $-D$ is a positive (effective) divisor, then the faisceau Ω^r_D consists of germs of meromorphic r-forms which vanish on D.

If there exists a meromorphic function α on M such that $(\alpha) = D_1 - D_2$, we say that the divisors D_1 and D_2 are linearly equivalent and we write $D_1 \approx D_2$. If $D_1 \approx D_2$, we have the canonical isomorphism $\Omega^r_{D_1} \approx \Omega^r_{D_2}$ and therefore

$$H^s(M, \Omega^r_{D_1}) \approx H^s(M, \Omega^r_{D_2}). \tag{3.1}$$

Hence

$$a^r_{D_1}(M) \approx a^r_{D_2}(M) \tag{3.2}$$

and, if M is compact Kähler, we have by (2.9)

$$a(D_1) = a(D_2) \tag{3.3}$$

if $D_1 \approx D_2$.

Now let S, T be irreducible, non-singular subvarieties of M of dimension $n - 1$, and suppose that $S \cdot T$ is irreducible and non-singular. We denote by $\Omega^r(S + T)$ the faisceau of the germs of holomorphics r-forms on the union of S and T, and by $\Omega^r(S \cdot T)$ the faisceau of the germs of holomorphic r-forms on $S \cdot T$. Let $p \in S \cdot T$. If $\alpha \in \Omega^r(S + T)_p$, then $\alpha = \beta + \gamma$ where β is a holomorphic r-form in a neighborhood of S at p, γ a holomorphic r-form in a neighborhood of T at p, such that $(\beta)_{S \cdot T} = (\gamma)_{S \cdot T}$. We have the exact sequence

$$0 \to \Omega^r(S + T) \xrightarrow{i} \Omega^r(S) + \Omega^r(T) \xrightarrow{k} \Omega^r(S \cdot T) \to 0. \tag{3.4}$$

In fact, if $\alpha \in (\Omega^r(S) + \Omega^r(T))_p$, $p \in S \cdot T$, then $\alpha = \beta + \gamma$ where $\beta \in \Omega^r(S)_p$, $\gamma \in \Omega^r(T)_p$, $(\beta - \gamma)_{S \cdot T} \in \Omega^r(S \cdot T)$, $(\beta - \gamma)_{S \cdot T} = 0$ if and only if α belongs to the image of $\Omega^r(S + T)_p$. Thus $k(\alpha) = (\beta - \gamma)_{S \cdot T} = 0$ if and only if α belongs to the image of $\Omega^r(S + T)_p$. Moreover, it is seen that the homomorphism k is onto.

The exact cohomology sequence corresponding to (3.4) is

$$\ldots \to H^s(S + T, \Omega^r) \xrightarrow{i^*} H^s(S, \Omega^r) + H^s(T, \Omega^r) \xrightarrow{k^*} H^s(S \cdot T, \Omega^r) \xrightarrow{\delta^*} H^{s+1}(S + T, \Omega^r) \to \ldots$$

Hence

$$\begin{aligned} \dim H^s(S + T, \Omega^r) = {} & \dim H^{r,s}(S) + \dim H^{r,s}(T) - \dim H^{r,s}(S \cdot T) + \\ & \dim \{H^{r,s-1}(S \cdot T)/k^*[H^{r,s-1}(S) + H^{r,s-1}(T)]\} + \\ & \dim \{H^{r,s}(S \cdot T)/k^*[H^{r,s}(S) + H^{r,s}(T)]\}. \end{aligned} \tag{3.5}$$

Multiplying both sides of (3.5) by $(-1)^s$ and summing on s, we obtain (cf. (1.11))

$$a^r(S + T) = a^r(S) + a^r(T) - a^r(S \cdot T). \tag{3.6}$$

Next, in a neighborhood of a point $p \in S \cdot T$, we can introduce local analytic coordinates $z^1_p, \ldots, z^n_p$ on M such that the minimal local equations for $S + T$ is $R_p(z) = z^1_p z^2_p$ where S is given locally by $z^1_p = 0$, T by $z^2_p = 0$. Given a form

$$\varphi = \sum_{i_1 < \ldots < i_s} \varphi_{i_1 \ldots i_s} dz^{i_1} \ldots dz^{i_s},$$

we define ν_p as in Section 1 if $p \in S + T$, $p \notin S \cdot T$, while for $p \in S \cdot T$ we define

$$\nu_p\varphi = \begin{cases} \sum \varphi_{1 \ldots i_n} dz^{i_2} \ldots dz^{i_n} & \text{in } U_p \cap S \\ -\sum \varphi_{i_1 2 i_3 \ldots i_n} dz^{i_1} dz^{i_3} \ldots dz^{i_n} & \text{in } U_p \cap T \end{cases}$$

If τ_p is a meromorphic r-form in U_p, $(\tau_p) + S + T \geq 0$, and if ψ is an arbitrary form of type $(n - r, n - 1)$ and of class C^∞ with carrier contained in a compact subdomain of U_p, then τ_p (or a current of type $(r, 0)$) satisfies (1.1) with

$$\mathfrak{R}_p[\psi] = \int_{S+T} \{\nu_p(R_p\tau_p)/\partial R_p \cdot \psi + (-1)^r (R_p\tau_p)/\partial R_p \cdot \nu_p\psi\}$$

where R_p is a minimal local equation for $S + T$ at p and where

$$\partial R_p = \begin{cases} 1 & p \in S + T, \quad p \notin S \cdot T \\ \partial R_p/\partial z_p^1 & \text{on } S, \quad p \in S \cdot T \\ \partial R_p/\partial z_p^2 & \text{on } T, \quad p \in S \cdot T. \end{cases}$$

Let

$$\mathcal{P}(\tau_p) = (\nu_p \cdot R_p\tau_p)/\partial R_p, \; \mathcal{Q}(\tau_p) = (R_p\tau_p)/(h_p \cdot \partial R_p),$$

where $\{h_p\}$ is a system which defines the characteristic divisor D of $S + T$. That is $\{h_p\} = \{h_p(S)\} + \{h_p(T)\}$ where $\{h_p(S)\}$ defines the characteristic divisor $D(S)$ of S, $\{h_p(T)\}$ the characteristic divisor $D(T)$ of T, $D = D(S + T) = D(S) + D(T)$. Then

$$\mathfrak{R}_p[\psi] = \int_{S+T} \{\mathcal{P}(\tau_p)\psi + (-1)^r \mathcal{Q}(\tau_p) \cdot \nu_p\psi\}$$

where

$$\mathcal{P}(\tau_p) \in \Omega^r_{S \cdot T}{}^{-1}(S + T), \; \mathcal{Q}(\tau_p) \in \Omega^r_{S \; T + D}(S + T).$$

Therefore, if we denote by $\mathfrak{R}^r_{S+T}$ the faisceau of the germs of all residues $\mathfrak{R}_p$, we have the canonical isomorphism

$$\mathfrak{R}^r_{S+T} \approx \Omega^r_{S \cdot T}{}^{-1}(S + T) + \Omega^r_{S \cdot T + D}(S + T) \tag{3.7}$$

We have the exact sequence (cf. (1.3))

$$0 \to \Omega^r \xrightarrow{i} \Omega^r_{S+T} \xrightarrow{\delta} \mathfrak{R}^r_{S+T} \to 0. \tag{3.8}$$

Therefore (cf. (1.10))

$$\begin{aligned} \dim H^s(M, \Omega^r_{S+T}) = {} & \dim H^s(S + T, \Omega^r_{S \cdot T}{}^{-1}) + \dim H^s(S + T, \Omega^r_{S \cdot T + D}) - \\ & \dim H^{r, s+1}(M) + \dim \{H^{r, s}(M)/\delta^* H^{s-1}(M, \mathfrak{R}^r_{S+T})\} + \\ & \dim \{H^{r, s+1}(M)/\delta^* H^s(M, \mathfrak{R}^r_{S+T})\}, \end{aligned} \tag{3.9}$$

and (cf. (1.11))

$$a^r_{S+T}(M) = a^r_{S \cdot T}{}^{-1}(S + T) + a^r_{S \cdot T + D}(S + T) + a^r(M). \tag{3.10}$$

4. Let M and S be as in Section 1, and let Φ_S^r be the faisceau of the germs of meromorphic r-forms which are closed under d in $M - S$ and which are multiples of $-S$. Let $\tau_p \in (\Phi_S^r)_p$, $p \in S$, and write $\tau_p = \sigma_p/z_p^1$ where $z_p^1 = 0$ on S. Then σ_p is holomorphic in a neighborhood of p, and

$$\tau_p = \sigma_{1p}/z_p^1 + \sigma_{2p}/z_p^1$$

where σ_{1p} is the sum of all terms involving dz_p^1, σ_{2p} the remaining terms. We have

$$0 = d\tau_p = d\sigma_p/z_p^1 - dz_p^1 \cdot \sigma_{2p}/(z_p^1)^2,$$

and therefore

$$\sigma_{2p} = z_p^1 \cdot d\sigma_p.$$

It follows that $\mathfrak{Q}(\tau_p) = 0$, and that

$$\mathfrak{R}_S^r \approx \Phi^{r-1}(S) \tag{4.1}$$

where $\Phi^{r-1}(S)$ is the faisceau of germs of holomorphic $(r-1)$-forms on S which are closed under d.

We have the exact sequence

$$0 \to \Phi^r \xrightarrow{i} \Phi_S^r \xrightarrow{d} \mathfrak{R}_S^r \to 0 \tag{4.2}$$

where Φ^r denotes the faisceau of the germs of holomorphic r-forms on M closed under d, and hence

$$\begin{aligned}\dim H^s(M, \Phi_S^r) = {} & \dim H^s(S, \Phi^{r-1}) - \dim H^{s+1}(M, \Phi^r) + \\ & \dim \{H^s(M, \Phi^r)/\delta^* H^{s-1}(S, \Phi^{r-1})\} + \\ & \dim \{H^{s+1}(M, \Phi^r)/\delta^* H^s(S, \Phi^{r-1})\}. \end{aligned} \tag{4.3}$$

Now let

$$B^{r,s,t} = \sum_{0 \le i \le t} A^{r+i, s+t-i},$$

and let $\Phi^{r,s,t}$ be the subfaisceau of $B^{r,s,t}$ formed by the d-closed germs. We have the exact sequence[1]

$$0 \to \Phi^{r,s,t} \xrightarrow{i} B^{r,s,t} \xrightarrow{d} \Phi^{r,s,t+1} \to 0,$$

and from the corresponding exact cohomology sequence we obtain the canonical isomorphism

$$H^q(M, \Phi^{r,s,t}) \approx K^{r,s,q+t}(M) \tag{4.4}$$

where

$$K^{r,s,t}(M) = H^0(M, \Phi^{r,s,t})/dH^0(M, B^{r,s,t-1}). \tag{4.5}$$

If M is a compact Kähler manifold, then

$$K^{r,s,t}(M) \approx \sum_{0 \leq i \leq t} H^{r+i,s+t-i}(M)$$

Substituting from (4.4) into (4.3), we obtain the formula

$$\begin{aligned}\dim H^s(M, \Phi_S^r) = {} & \dim K^{r-1,s}(S) - \dim K^{r,s+1}(M) + \\ & \dim \{K^{r,s}(M)/\delta^* K^{r-1,s-1}(S)\} + \\ & \dim \{K^{r,s+1}(M)/\delta^* K^{r-1,s}(S)\}, \quad (4.6)\end{aligned}$$

where, for simplicity, we have written $K^{r,s} = K^{r,0,s}$.

Let

$$b^r(M) = \sum_{s=0}^{n} (-1)^s \dim H^s(M, \Phi^r),$$

$$b_S^r(M) = \sum_{s=0}^{n} (-1)^s \dim H^s(M, \Phi_S^r).$$

Then (cf. (1.11))

$$b_S^r(M) = b^{r-1}(S) + b^r(M). \quad (4.7)$$

Given a locally finite covering $\mathfrak{U}$ of M, we can describe the homomorphism

$$H^q(M, \Phi^{r,s,t}) \to K^{r,s,q+t}(M) \quad (4.8)$$

as follows. Given $\varphi \in Z^q(\mathfrak{U}, \Phi^{r,s,t})$, then $\varphi \in Z^q(\mathfrak{U}, B^{r,s,t})$ and, since $B^{r,s,t}$ is a fine faisceau, $H^q(\mathfrak{U}, B^{r,s,t}) = 0$, $q > 0$. Hence there exists $\eta_0 \in C^{q-1}(\mathfrak{U}, B^{r,s,t})$ such that

$$\varphi = \delta\eta_0.$$

We define

$$\sigma_1 = d\eta_0, \qquad \sigma_1 \in Z^{q-1}(\mathfrak{U}, \Phi^{r,s,t+1})$$

and so on. Finally, we obtain $\sigma_q \in Z^0(\mathfrak{U}, \Phi^{r,s,q+t}) = \Gamma(\Phi^{r,s,q+t})$, and (4.8) is defined by $\varphi \to \sigma_q$.

Given $\omega \in Z^s(\mathcal{U}(S), \Phi^{r-1})$, let $\tau = \kappa \circ \omega$; then $\delta\tau \in Z^{s+1}(\mathfrak{U}, \Phi^r)$ and we may take $\eta_0 = \tau$. In this case (cf. (2.4))

$$\sigma_1[\psi] = 2\pi i \int_S \omega\psi. \quad (4.9)$$

Assume that M is compact Kähler. Then

$$K^{r,s}(M) = K^{r,0,s}(M) \approx \sum_{0 \leq i \leq s} H^{r+i,s-i}(M), \quad (4.10)$$

and

$$K^{r,s+1}(M)/\delta^* K^{r-1,s}(S) \approx \{\psi \mid \psi \in K^{r,s+1}(M), (\sigma_{s+1}, \psi) = 0\}.$$

Hence

$$\dim H^s(M, \Phi_S^r) = \dim K^{r-1,s}(S) - \dim K^{r,s+1}(M) + \dim \{\psi \mid \psi \in K^{r,s}(M), \quad (\sigma_s, \psi) = 0\} + \dim \{\psi \mid \psi \in K^{r,s+1}(M), (\sigma_{s+1}, \psi) = 0\}. \quad (4.11)$$

Returning to the general case, we consider the exact sequence

$$0 \to \Phi^{r-1} \xrightarrow{i} \Omega^{r-1} \xrightarrow{\delta} \Phi^r \to 0 \quad (4.12)$$

and the corresponding cohomology sequence

$$\to H^s(M, \Omega^{r-1}) \xrightarrow{\delta^*} H^s(M, \Phi^r) \xrightarrow{\delta^*} H^{s+1}(M, \Phi^{r-1}) \xrightarrow{i^*} H^{s+1}(M, \Omega^{r-1}) \to.$$

Let $u \in H^s(M, \Phi^r)$. If $\delta^* u = 0$, then $u \in \delta^* H^s(M, \Omega^{r-1})$; that is, there is a covering $\mathfrak{U}$ and a $\varphi \in Z^s(\mathfrak{U}, \Phi^r)$, $\pi(\mathfrak{U}) \cdot \varphi = u$, such that $\varphi = \delta\omega$, $\omega \in Z^s(\mathfrak{U}, \Omega^{r-1})$. Let $\tau_s \in \Gamma(F^{r-1,s}, M)$ be the image of ω in the process describing (2.3). Then $\sigma_s = \delta\tau_s$ is the image of $\delta\omega$ in the same process. If M is compact Kähler, we conclude that $\varphi = \delta\psi$, $\psi \in C^{s-1}(\mathfrak{U}, \Omega^r)$ (cf. ref. 4).

The above remarks are merely intended to suggest the manner in which cohomology with coefficients in a faisceau may be applied to obtain Riemann-Roch theorems. A systematic development will be presented elsewhere.

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IMAGE THEORY OF SUPERPOSED VECTOR SETS

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Introduction.—There has recently been great interest in the solution of Patterson syntheses. The writer's theory of solving these syntheses[1–4] is based upon a theorem concerned with the location of image polygons, which may be stated as follows: *The locations of a complete set of image polygons is the complete inverse solution.*

This theorem follows from a direct interpretation of the vector set matrix. An alternative route of attaining an equivalent solution is through the use of superposed vector maps. This was the general method followed by Clastre and Gay,[5] Garrido,[6] and McLachlan.[7–11] Their results were derived from various analytical treatments. Image theory can also be ap-

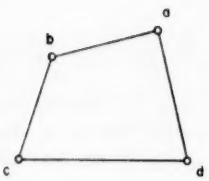
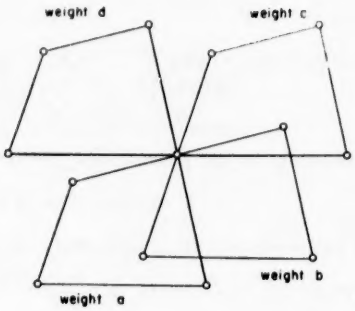
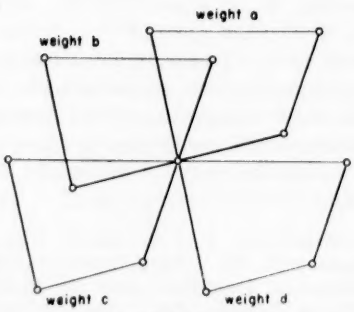


FIGURE 1





FIGURES 2 (TOP) AND 3

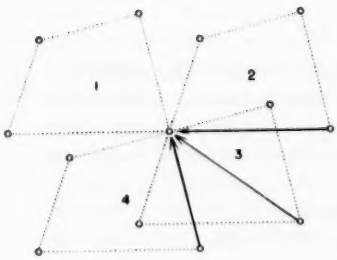


FIGURE 4

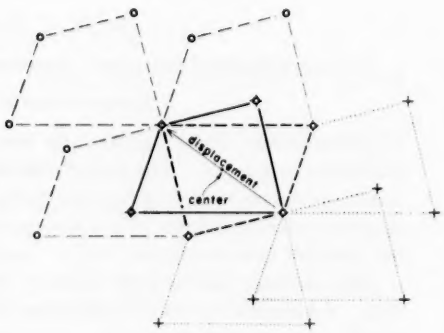


FIGURE 5

plied to superposed vector sets with profitable results. This theory is outlined here. It predicts solution functions and their relation to the electron density, a relation which is treated in an accompanying paper.

Weighted Images.—An elementary result of image theory is that a vector set which is based upon a fundamental set of n points, figure 1, can be resolved into n identically shaped n-gons, figure 2, each of which is the required solution. Alternatively, it can be resolved into n inverse n-gons, figure 3. If the various points of the fundamental set have different weights, a, b, c, . . ., then these n solutions are differently weighted by factors a, b, c, . . ., figures 2 and 3. Each of these image polygons is attached to the origin by a different vertex. For convenience let the several polygons be numbered 1, 2, 3, . . . n, figure 4.

Superposed Vector Maps.—Consider two identical vector maps, A and B. Map B can be superposed on map A so that image 1_B superposes on any of the images 1_A, 2_A, 3_A, . . . n_A. It is evident that n total rational

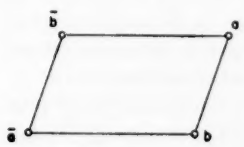


FIGURE 6

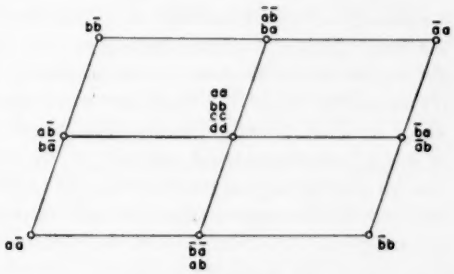


FIGURE 7

superpositions can be made. For superposition 1_B1_A, there is no relative displacement of maps A and B, and all images of the two maps superpose. On the other hand, when 1_B superposes on 2_A, 3_A, . . . n_A, then, in general, only one image of map A (and, as will appear, its inverse) superposes on one image of map B. This image (and its inverse) are shown in heavy lines in figure 5. For each polygon in A, therefore, there are $n-1$ ways of effecting a nonidentical superposition by shifting B, or $n(n-1)$ ways in all. (Another analysis of this is as follows: There are $n(n-1)$ non-origin points in B. When any of these is superposed on the origin of A, a vertex of one B polygon is brought to the corresponding vertex at the origin for an A polygon.)

Centrosymmetry of a Superposed Pair of Maps.—The origin of all vector sets contains an inversion center. The combination of an inversion, followed by any translation, generates another inversion center located halfway along the translation. It follows that if a pair of maps, A and B, are given any relative displacement whatever, the pair contains a *displacement*

inversion center halfway along the line of displacement. If the displacement is a rational one (i.e., one that causes a superposition of a pair of image polygons) this requires the superposition of the particular inverse polygons related to the superposed polygons by this displacement inversion, figure 5.

If the fundamental set is centrosymmetrical, figure 6, then the complete solution polygon is centrosymmetrical, figure 7. In this case the polygon and its inverse coincide. This implies that for centrosymmetrical fundamental sets, a displacement of a pair of maps which produces a rational superposition does not produce a separate superposition of the inverse polygon. If the displacement is an appropriate one, those points of the two vector sets which superpose thus consitute the points of the fundamental set alone, so that a single appropriate displacement can effect a complete solution of the vector set. This is Clastre and Gay's[5] and Garrido's[6] primary result.

It should be noted that although $n(n-1)$ possible displacements can effect a rational superposition, only $n-1$ are directions which provide a single solution. This is because centrosymmetry gives rise to a degeneracy in the vector set in the form of a coincidence in pairs of all unsymmetrical vector points, figure 7. There are n symmetrical points in such a set (of which $n-1$ are nonorigin points) like $a\bar{a}$, $b\bar{b}$, etc., whereas there are $n^2-n = n(n-1)$ unsymmetrical ones like ab, $\bar{b}a$, etc. The displacement of B so that an unsymmetrical point is brought to the origin of A, actually brings two coincident unsymmetrical points to the origin of A and therefore causes two images of B to coincide with the corresponding two images of A. This gives rise to two displaced solutions. If such a double solution is reached, and can be recognized, it can be eliminated by using a third map, C, and giving it a different rational displacement. The triple coincidence of points in A, B, and C is a single solution polygon.

Elimination of the Inverse Solution.—The inverse solution which arises with a non-centrosymmetrical fundamental set can be eliminated by the use of a third map, C, of the vector set. As pointed out above, B can be displaced in $n(n-1)$ ways to provide a superposition of one solution polygon plus its inverse in A and B. A third map, C, can be displaced in $n(n-2)$ ways to superpose with an image in A and yet not be undisplaced with respect to either A or B. But once a polygon in A has been chosen for a superposition by one in B, then B can be superposed on A in only $n-1$ ways, figure 4. If C is not to be displaced in exactly the same way as B, then only $n-2$ displacements are open to it such that the same polygon in A is covered, figure 4.

Since B and C are displaced in different ways, the locations of the displacement inversion centers for the pairs AB and AC are different. This requires that the particular inverse polygon which superposes for the pair

AB is different from the particular inverse polygon which superposes for the pair AC. Thus, when A, B, and C are superposed, using two different displacements to form a triple superposition, the inverses do not fall in triple superposition. This shows that a vector set can be solved so that there is no interference from the inverse solution, by finding superpositions due to two *appropriate* displacements.

More than three vector maps can be superposed. If there are n points in the fundamental set, there are n images of the solution. Therefore n different images of n different maps can be superposed. The pth map can be superposed on the selected image of map A in $n-p$ ways, none of which is a displacement already utilized for other maps.

The outline just given of the theory of superposed vector sets does not necessarily reveal the practical application of the theory to the solution of Patterson syntheses, particularly with regard to the practical selection of displacement directions. The practical features are, perhaps, more readily determined by using image-locations theory.[4] The theory does open the way to a discussion of solution functions for solving Patterson syntheses.

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SOLUTION FUNCTIONS FOR SOLVING SUPERPOSED PATTERSON SYNTHESES

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Introduction.—In the writer's original image-location theory,[1-4] the Patterson syntheses is solved by means of *image-seeking functions*, which seek and find image locations in the Patterson synthesis and which bear a definite relation to the electron density of the crystal. In the theory of superposed vector sets, functions corresponding to image-seeking functions can be devised. These may be termed *solution functions*.

Each separate image in the Patterson synthesis can be regarded as a solution of the electron density of the crystal, scaled by a weighting factor

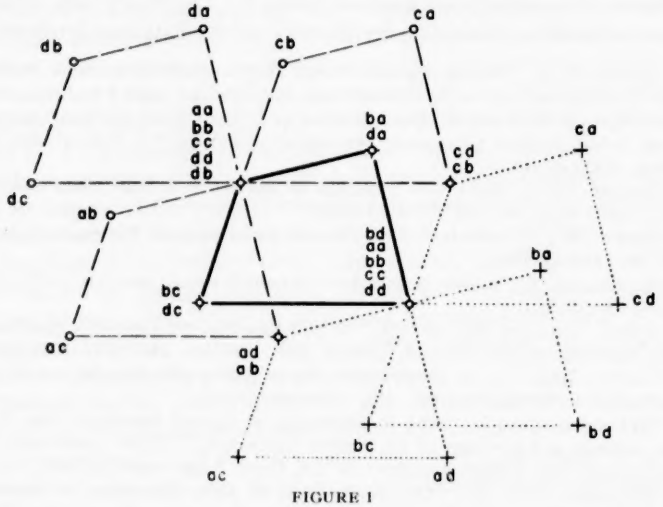

FIGURE 1

which is dependent on the density of the atom responsible for that image. From this it is evident that various functions of the superposed images should bear a close relation to the electron density. In increasing similarity to the electron density there should exist the equivalent of the following image-seeking functions: sum function, product function, and minimum function.

Sum Function.—If one merely adds together the values at each superposed point of the displaced Patterson maps, thus forming a sum function

of the displaced Patterson values, the contrast of the superposed polygons is enhanced over those which have not superposed, figure 1. This gain in contrast is not very great, amounting to a factor crudely equal to the number of superpositions. If the atoms in the crystal have weights and designations $a, b, c, d, \ldots$, then the atoms of the images have weights as shown in the lines of the following array:

$$\begin{array}{cccccc} aa & ab & ac & ad & . . & . . \\ ba & bb & bc & bd & . . & . . \\ ca & cb & cc & cd & . . & . . \\ da & db & dc & dd & . . & . . \\ . . & . . & . . & . . & . . & . . \\ . . & . . & . . & . . & . . & . . \end{array} \qquad (1)$$

If all n possible maps are displaced so that all these images superpose, and the values of the Patterson function added over the entire map area, the atoms of the superposed images are represented by the values

$$(a+b+c+d+\ldots)a \quad (a+b+c+d+\ldots)b$$
$$(a+b+c+d+\ldots)c \quad (a+b+c+d+\ldots)d \ldots (2)$$

whereas the atoms of the polygons which did not superpose have weights

$$aa \quad ab \quad ac \quad ad \quad . \quad . \qquad (3)$$

etc. This means that there is a heavy background of many images like (3) in which solution (2) is embedded, figure 1. Since (2) is simply $a, b, c, d, \ldots$, scaled by factor $(a + b + c + d \ldots)$, the sum function is the scaled electron density embedded in a high background. Let $S_p(xyz)$ denote the sum of p displaced Patterson syntheses. Then the relation of the sum function to the electron density is

$$S_p(xyz) \geq K_1 \rho(xyz), \qquad (4)$$

where K_1 is a scale factor depending on weighting $a, b, c, \ldots$. The inequality not only allows for false background contributed by non-superposed polygons, but also for the possibility that even before superposition, the peaks of some of the atoms of the polygon may have been multiple, i.e., unresolved atom pairs. The strength of inequality (4) is evidently increased by increasing the number of superpositions.

The sum function has been used by McLachlan[6, 7] in solving Patterson syntheses. McLachlan used the function somewhat intuitively, and without relating it specifically to the electron density as in (4).

Product Function.—If, instead of adding superposed values of the n displaced Patterson syntheses, one forms their products, the resulting function of the Patterson values is called the *product function*. At image superpositions the function has the value

$$(a \times b \times c \times d \ldots)a^n \quad (a \times b \times c \times d \ldots)b^n$$
$$(a \times b \times c \times d \ldots)c^n \quad (a \times b \times c \times d \ldots)d^n \ldots (5)$$

Since this is simply $(a, b, c, d, \ldots)^n$, scaled by the factor $(a \times b \times c \times d \ldots)$, it is evident that the nth root of the product function provides the scaled equivalent of the electron density embedded in a possible background, figure 1. This background is smaller than that for the sum function, since the absence of even one image gives the product function an ideal value of zero. Actually, this background is appreciable since any superposition of non-zero Patterson values in the different maps produces a non-zero product and the Patterson synthesis generally has a high background. If $\Pi_p(xyz)$ represents the product, at coordinates xyz, of p superposed Patterson syntheses, then the relation of the product function to the electron density evidently is

$$\sqrt[p]{\Pi_p(xyz)} \geq K_2\rho(xyz). \qquad (6)$$

The inequality aspect of (6) not only allows for non-zero value of the function which occurs when non-zero background superposes on an image, but also allows for enhanced values of Π which arise when one or more of the superposed Patterson peaks are part of an unresolved group of peaks.

The product function has been used by McLachlan[5, 6] to give a qualitative picture of the crystal structure from Patterson syntheses. The relation of the product function to the electron density was pointed out by Buerger,[1-4] and applied by him to give approximations to the electron density.[4]

Minimum Function.—The minimum function finds superpositions of p images by rejecting any set of superposed images when even one image is missing. To see this, consider first a simplified version of a minimum function which can be designated the *unscaled minimum function*, ${}^{U}M_p(xyz)$.

To form the unscaled minimum function one scrutinizes the p superposed Patterson values at point xyz and records the minimum value of this group of p values. Thus, at the locations of the superposed images in the columns of (1), one would record, say,

$$ca \quad cb \quad cc \quad cd \quad . \quad ., \qquad (7)$$

assuming that c is the least of the weights $a, b, c, d, \ldots$. Since (7) is a set of values which is the same as the electron densities of the solution, scaled by factor c, it is evident that the unscaled minimum function is ideally proportional to the electron density. If account is taken of chance superpositions in the Patterson syntheses, as before, an inequality relation with the electron density is valid, namely:

$$M_p(xyz) \geq K_3\rho(xyz). \qquad (8)$$

This function isolates the solution polygon better than the sum function or the product function, because the background of the function is always the minimum of the p superposed Patterson syntheses, figure 1. For, even if $p - 1$ of the p images accidentally superpose, the value of the function at such chance superpositions is merely Patterson background. Thus the function isolates the solution embedded in Patterson background.

A disadvantage of the unscaled type of minimum function is that the particular image of least weight is preserved. This may be very little above the general Patterson background. A superior type of minimum function can be formed after first scaling the various superposed Patterson syntheses so that the superposed image polygons have the same weight. For example, let $p - 1$ maps be scaled up so that all images have the weight of the image of greatest weight. When this is done the minimum function preserves the images of greatest weight in the background of least weight. This is the minimum function as originally defined,[3, 4] and when it is necessary to distinguish it from the unscaled minimum function ${}^{U}M_p(xyz)$, it may be identified by the superscript thus: ${}^{S}M_p(xyz)$. Actually, in the use which has been made of the minimum function so far, no scaling has been necessary since the function has always been used with images caused by sets of atoms of the same chemical species. The scaled minimum function has the advantage that images of all weights have equal control over finding the superposed image, as well as giving a solution image having maximum contrast over background, as pointed out above.

Application of Solution Functions to Solving Difference-Patterson Maps.—The writer has shown that when isomorphous pairs of crystals are available, a difference-Patterson map can be prepared which has the important property that its peaks occur only at the ends of vectors originating at the replaced atom positions.[8] If the cell of the crystal contains n atoms and r are replaced in the isomorphous pair, then the ordinary Patterson map contains n^2 peaks, whereas the difference Patterson contains only rn peaks. In image language, the ordinary Patterson contains n images of the solution (plus inverse solutions if the crystal lacks an inversion center), whereas the difference Patterson contains only r images of the solution (plus r inverse solutions in case the replaced atoms do not occupy inversion centers).

In solving difference Pattersons, two cases arise. If the locations of the r replaceable atoms are known (say because of special position considerations, or because the locations of these atoms are revealed by the Patterson itself), the relative displacements required to bring together these r images are known. But even if the locations of the replaced atoms are unknown, it is possible that the image motif and its duplication can be recognized in the difference Patterson, since there are only r images. In either case, the appropriate displacement of duplicate difference-Patterson maps followed by application of a solution function, preferably the minimum function, serves to solve the crystal structure.

Rogers has suggested a Fourier scheme for accomplishing this superposition of the r images of the difference Patterson.[9] While the justification of Rogers' method is not clear, it appears to have the properties of the sum function. It therefore suffers from the intolerable background of the sum function. It is also much more difficult to apply than the method described above.

[1] Buerger, M. J., "The Application of Image Theory to Crystal Structure Analysis" (part of conference held at the Pennsylvania State College, April 6–8, 1950, entitled "Computing Methods and the Phase Problem in X-Ray Crystal Analysis," published by the X-Ray Analysis Laboratory, Department of Physics, The Pennsylvania State College, 1952), pp. 43–56.

[2] Buerger, M. J., "Some New Functions of Interest in X-Ray Crystallography," PROC. NATL. ACAD. SCI., **36,** 376–382 (1950).

[3] Buerger, M. J., "Limitation of the Electron Density by the Patterson Function," *Ibid.*, **36,** 738–742 (1950).

[4] Buerger, M. J., "A New Approach to Crystal-Structure Analysis," *Acta Cryst.*, **4,** 531–544 (1951).

[5] McLachlan, Dan, Jr., "The Determination of Crystal Structures from X-Ray Data without a Knowledge of the Fourier Coefficients." Paper presented at the Pennsylvania State College meeting of the Am. Cryst. Assoc., April 10, 1950.

[6] McLachlan, Dan, Jr., "The Determination of Crystal Structures from X-Ray Data without a Knowledge of the Phases of the Fourier Coefficients," PROC. NATL. ACAD. SCI., **37,** 115–124 (1951).

[7] Thomas, Ivor D., and McLachlan, Dan, Jr., "Some Critical Tests of the Use of Mixed Projections in Crystal-Structure Determinations," *Acta Cryst.*, **5,** 301–306 (1952).

[8] Buerger, M. J., "A New Fourier Series Technique for Crystal Structure Determination," PROC. NATL. ACAD. SCI., **28,** 281–285 (1942).

[9] Rogers, D., "New Methods of Direct Structure Determination Using Modified Patterson Maps," *Research*, **4,** 295–296 (1951).

AN INTERSECTION FUNCTION AND ITS RELATIONS TO THE MINIMUM FUNCTION OF X-RAY CRYSTALLOGRAPHY

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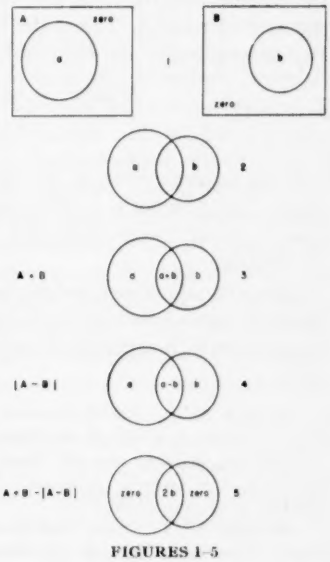
Introduction.—Several functions have been devised to solve Patterson syntheses,[1–4] namely the sum function, product function, and minimum function. A simple discussion of these is given in an accompanying paper.[5] These are functions of Patterson values, and they bear inequality relations with the electron density. The weakest inequality is provided by the sum function, the strongest by the minimum function.

Sum Function.—Consider two Patterson syntheses, A and B, figure 1,

on which occur areas of uniform density a and b respectively, each surrounded by areas of zero density. When Patterson syntheses A and B are superposed, let these two density areas partially overlap, figure 2. For definiteness, suppose $b < a$.

Now consider the sum function of this displaced pair. The values of the function in the various areas of the superposed pair are shown in figure 3. Values a and b occur outside the common area, while $a + b$ occurs in the common area. It is evident from the accompanying paper[5] that a characteristic of a desirable solution function is that it should find the common part, or intersection, of the two areas of maps A and B. The sum function fails in this respect since it finds not only this area but false areas as well. If the sum function could be corrected for the non-overlapped parts of the areas of levels a and b in figure 3, the corrected function would be an intersection function. It is evident that such a correction could be provided by subtracting the absolute values of the difference of maps A and B.



FIGURES 1–5

An Intersection Function.—Let $S_{AB}(xyz)$ represent the sum of the superposed values on the displaced Patterson maps A and B; let $D_{AB}(xyz)$ represent the absolute differences of these values. Then the values of these functions in the three kinds of areas of figure 2, and their differences, are

		Value of Function in			
	Function	Left Non-common Area	Common Area	Right Non-common Area	
(Fig. 3)	$S_{AB}(xyz)$	a	$a + b$	b	(1)
(Fig. 4)	$D_{AB}(xyz)$	a	$a - b$	b	(2)
(Fig. 5)	$S_{AB}(xyz) - D_{AB}(xyz)$	0	$2b$	0	(3)

Thus, the common area can be found by a combination of the sum and difference function:

$$\mathrm{Int}_{AB}(xyz) = S_{AB}(xyz) - D_{AB}(xyz) \qquad (4)$$

$$= A + B - |A - B|. \tag{5}$$

Here A and B are the values of the Patterson function at superposed points of the two appropriately displaced Patterson syntheses.

Relation to Minimum Function.—Examination of figures 2–5 shows that the intersection function has exactly twice the value of the minimum function of rank[5] 2. Thus the intersection function furnishes an alternative description of the minimum function. One concludes that

$$M_2(xyz) = {}^1/_2 \,\mathrm{Int}(xyz) \tag{6}$$

$$= {}^1/_2 \,(A + B - |A - B|) \tag{7}$$

If the analysis given in (1), (2), and (3) is repeated using (3) and a new map, C, containing a non-zero area of level c (either greater or less than level b), it becomes evident that relation (6) is true for minimum functions of all ranks.

The original manner of deriving the minimum function[3] was such that it was not obvious that it was a common-ground function. Its discussion in connection with superposed vector sets[5] makes such an identification possible.

[1] Buerger, M. J., "The Application of Image Theory to Crystal Structure Analysis" (part of conference held at the Pennsylvania State College, April 6–8, 1950, entitled "Computing Methods and the Phase Problem in X-Ray Crystal Analysis," published by the X-Ray Analysis Laboratory, Department of Physics, The Pennsylvania State College, 1952), pp. 43–56.

[2] Buerger, M. J., "Some New Functions of Interest in X-Ray Crystallography," PROC. NATL. ACAD. SCI., **36,** 376–382 (1950).

[3] Buerger, M. J., "Limitation of the Electron Density by the Patterson Function," *Ibid.*, **36,** 738–742 (1950).

[4] Buerger, M. J., "A New Approach to Crystal-Structure Analysis," *Acta Cryst.*, **4,** 531–544 (1951).

[5] Buerger, M. J., "Solution Functions for Solving Superposed Patterson Syntheses," PROC. NATL. ACAD. SCI., **39,** 674–678 (1953).

ABOUT SPACE-TIME ASYMMETRY IN THE REALM OF CLASSICAL GENERAL AND CRYSTAL PHYSICS

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Like thermodynamics and dimensional analysis, symmetry considerations enable one to make predictions without detailed knowledge of the object. At the end of the last century, P. Curie and W. Voigt tried to apply this method, which had proved to be so successful in the morphology of crystals, to physics. Their endeavor was only partially successful. The reason for this fact may be that these great crystal physicists have not adapted and generalized the meaning of symmetry to suit the new field. The mere application of the existing spatial symmetry concept could not be satisfactory, partly because in most physical problems the consideration of asymmetry[1] is more useful than that of symmetry, but principally because most physical problems, in contrast to the morphology of crystals, are concerned not only with spatial structure but also with its relation to time, i.e., movement.

The generalization of the symmetry concept which is necessary for the transition from the morphology of crystals to physics consists in considering transformations involving the time axis in addition to spatial transformations. Symmetry in space is the identity, for the description of a structure, of different coordinate systems which can be obtained by transformations of the space coordinates. Symmetry in relation to time has to be the invariance with respect to transformations of the time coordinate. The transformation may consist in a shifting ($t' = t + t_0$), or in an inversion of time ($t' = -t$), which may be denoted also as reversal of time or as a time reflection. The time inversion is the same operation as letting a movie film run backward. The act of inversion is not a physical act, but the study of the opposite chronological order of the same items. E. Wigner[2] was the first to recognize time transformations as symmetry operations and to apply this kind of operator to problems of quantum mechanics.

In the following we shall be concerned with the inversion of time, not with the shifting of the time axis. We shall speak of time symmetry if the time inversion has no influence upon the sign of the quantity and of time asymmetry if the time inversion causes the sign of the quantity to be reversed. Quantities like linear or rotational velocities are time-asymmetric while a crystal structure is time-symmetric. Quantities related to movement may be time-symmetric, for instance those containing velocity to an even power, such as energy; or those containing acceleration, the second derivative.

In the following we shall consider only rotations and reflections in space and inversion of time but will exclude all space and time translations, as well as relativistic and quantum phenomena. Then we shall introduce the asymmetry concept, which applies both to time-symmetric and time-asymmetric quantities. We shall show that the concept of space-time asymmetry enables one to predict the possibility or impossibility of physical effects, such as pyro-electricity, pyro-magnetism, etc. There is, at present, no certainty about the existence of several such effects which appear to be possible from the point of view of classical crystal physics. In order to judge the possibility or impossibility of physical effects, we assume the axiom that in an equilibrium or in a stationary state no asymmetry can appear which does not exist already in the object or in the influence which is exerted upon it, or in the combination of both.

A physical assymmetry needs always the indication of the relation to time besides the spatial type of asymmetry. The same type of asymmetry exhibiting or not exhibiting invariance with respect to time inversion represent two essentially different asymmetries.

Asymmetry is much more than the counterpart of symmetry. Asymmetry indicates the existence of characteristic differences, whereas symmetry discards characteristic features. Certain physical phenomena are intrinsically related to certain types of asymmetry, whereas certain symmetry elements may exist without necessarily being related to those effects. "C'est la dissimetrie, qui crée le phenomene" stated P. Curie.[3]

The most important application of symmetry considerations in physics studied by P. Curie and W. Voigt is the establishment of the symmetry properties of the electric and magnetic field. Both authors came to the conclusion that the electric field must be considered as a polar vector, the magnetic field as an axial one. Voigt showed that one can conclude from Maxwell's equations that one of these fields must be a polar vector and the other an axial one. The result is obtained immediately by considering the transformation which consists in the multiplication of all space coordinates by the factor -1. Voigt writes Maxwell's equations in the form given by H. Hertz:

$$\delta A/\delta t = c(\delta Y/\delta z - \delta Z/\delta y); \quad \delta X/\delta t = c(\delta C/\delta y - \delta B/\delta z)$$

. . . . . .

. . . . . .

where X, Y, Z are the electric and A, B, C the magnetic field components. The decision that the electric field is the polar one is derived from the fact that crystals like tourmaline with polar shape (which is related to polar inner structure) possess an inner electric field. This field is revealed by heating (pyro-electricity) and by compression or dilation (piezo-electricity).

The fact that the magnetic field represents the axial vector can be derived from the Faraday effect which may be called a true circular[4] double refraction: the difference between the refractive indices for light the electric vector of which rotates in the direction of the field-producing current or in the opposite direction.[5]

Disregarding the relation to time, the electric field was assumed to have the same symmetry as linear velocity or polar (hemimorphic) crystals such as tourmaline. Similarly the symmetry of the magnetic field was assumed to be the same as that of angular velocity or circular (paramorphic) crystals, such as apatite, dolomite, etc. On the basis of such a classification one had to expect a series of new physical or crystal physical effects. However, experimental research did not confirm the conclusions arrived at; the expected effects were never found.

It was expected, for instance, that circularly asymmetric crystals could exhibit a constant magnetic moment, in analogy to the electric field in tourmaline. P. Curie[6] made various experiments in order to detect such inherent magnetism but failed to obtain any positive result. W. Voigt[7] gave a rather detailed report of his work to obtain such an effect and concluded that the existence of such an effect—which he called pyromagnetism—is probable. Up to date there does not seem to exist a valid consideration concerning the possibility of the "pyromagnetic" effect.[8]

The whole complex becomes clear, however, if one introduces the concept of asymmetry in relation to time. Applying the operation of time inversion to Maxwell's equations, one sees immediately that one of the two fields is time-symmetric and the other time-asymmetric. In order to decide whether the electric or the magnetic field is time-asymmetric, we consider the same examples as above, the pyro- and piezo-electric crystals and the Faraday effect. The electric field in the polar crystals must be time-symmetric like the crystal structure from which it arises. If we do not assume any further time asymmetry, the magnetic field reverses its sign with time inversion as the rotation of the electric vector of the light in the Faraday effect. This conclusion is to be drawn from the Faraday effect equally well as we derive the enantiomorphic structure of molecules in solution from their optical activity. Summing up, we can say that the electric field is space-asymmetric and time-symmetric, the magnetic field is space-symmetric and time-asymmetric if we call space symmetry and space asymmetry the invariance and the reversal of signs, respectively, under the operation of inversion of space. Having proved the difference in relation to the time we recognize that a magnetic moment cannot arise from a circular crystal structure. *Pyromagnetism and piezomagnetism cannot exist.*

There are other parts of physics in which fruitless experimental investigations could have been avoided by realizing the behavior of the magnetic

field with respect to time inversion. According to the theory of thermal and electrical conductivity of crystals developed by W. Thomson[9] and others, a circular asymmetry of the conductivity seemed to be quite possible. That is to say, the conductivity for clockwise and counterclockwise currents (seen from the same side of the crystal) could be different. Soret[10] and Voigt[11] tried to prove the existence of these "rotational coefficients" in careful experiments. They found no proof for the expected effect. Onsager[12] showed by a new theoretical treatment that such an effect could be expected only under the influence of an external magnetic field or of Coriolis forces.

The circularity of conductivity is a time-asymmetric property. Processes of conduction are not only time-asymmetric, they are irreversible. One direction of time corresponds to the probable course, the other is improbable according to the second law of thermodynamics. Potential differences are time-symmetric. The circular asymmetry consists in the difference of resistance against the clockwise and counterclockwise currents, being probable or not. These currents are naturally time-asymmetric. Hence, a crystal structure, which is time-symmetric, cannot cause a circularly asymmetric conductivity. Such a property can be found only in a system with time-asymmetric circularity, with a mechanical or electrical rotation, with Coriolis forces or magnetic fields. The Hall effect corresponds indeed to circular electric conductivity, the Righi-Leduc effect to a circular thermal conductivity, both produced by a magnetic field.

With the aid of the triasymmetry concept[13] it is possible to extend the above conclusion yet further. The time-asymmetric circularity may be produced by a combination of a time-asymmetric polarity with a time-symmetric enantiomorphism. In other words, circular conductivity may result from a polar conductivity and an enantiomorphic structure. Polar conductivity is a difference of conductivity in two opposite directions. It is evident that polarity of conductivity is a time asymmetric property too, possible only if the object under consideration exhibits a time asymmetric polarity: a linear movement like convection or diffusion of matter, or electric or thermal currents. Contrary to earlier work,[14] polar structure, as it exists in polar crystals such as tourmaline, cannot cause polarity of conductivity. Similarly polarity of electric conductivity and of diffusion cannot be the properties of polar crystals.

The so-called polar conductivity of dry and of electrolytic rectifiers represents no primary property. In the case of primary properties the effect is proportional to the influence ($i = K \cdot E$). If, however, in addition to the linear term, there is a quadratic one ($i = K_1 \cdot E + K_2 \cdot E^2$) the corresponding constant may be called a secondary property. It may appear on account of a change (chemical) in the object proportional to the influence. The effect is therefore proportional to the second power of the influence.

In systems of one crystalline phase only these secondary effects are rather small because an appreciable deformation of the crystal lattice needs very large forces.

In the following we try to make a more positive use of the asymmetry concept, predicting some possible effects. There must exist a new effect, analogous to the Hall effect, in which a circular crystal is substituted for the magnetic field. An electric potential difference applied perpendicularly to the circular axis of such a crystal will produce an electric field perpendicular to the plane through the circular axis and the direction of the applied field. This effect would show that the dielectric constant of merely circular crystals is not represented by a simple second-order tensor, as experiments to date appear to indicate. Molecular considerations appear to support the possibility of this effect.

Two triasymmetric effects can be obtained by means of a circular crystal. In an electric field parallel to the circular axis a circular crystal must acquire enantiomorphism. In this situation the crystal can be expected to show optical activity and mechanical contorsion.

Exerting an enantiomorphic force upon a circular crystal will give this crystal polarity. Hence torsion may produce electric polarization. The electricity produced by torsion could be called "strepho-electricity."

The above three effects are possible only in circular crystals. They may deserve some interest because circularity, in contrast to polarity and enantiomorphism, cannot be proved directly. Since circularity is the unique higher asymmetry compatible with a center of symmetry, it is the unique higher asymmetry which manifests itself in Laue diagrams.

The consideration of space-time asymmetry may prove to be useful outside the realm of crystal physics. The process of splitting a magnetic dipole into two free magnetic charges is seen to be impossible if one considers the space-time asymmetry involved in this process. Present-day literature is ambiguous concerning this point. Thus Dirac[15] discusses the reason why the separation of electric charges is so much easier than that of magnetic charges and Ehrenhaft believes to have succeeded in obtaining free magnetic charges.

As already stated, a magnetic momentum corresponds to a time asymmetric circularity and has therefore the character of a simple rotation. As Curie[16] already pointed out, a free magnetic pole would be enantiomorphic. He supposed therefore that optically active substances could possess free magnetism. However, the time asymmetry of the magnetic moment cannot be lost by the process of splitting. Otherwise, the recomposition of the free south and north poles would not furnish the original time-assymmetric moment. Hence, a free magnetic charge would be a time-asymmetric enantiomorphism, a time asymmetric "pseudoscalar" and not a time-symmetric "pseudoscalar" as the enantiomorphic structure of optically active substances is.

A time-asymmetric enantiomorphism occurs in the combination of electric and magnetic fields with parallel lines of force, as proposed by P. Curie[17] in order to produce optical activity in dielectrics. The time-asymmetry of this enantiomorphism, which is indeed a time-asymmetric triasymmetry, does not permit a time-symmetric optical activity. If a magnetic momentum is nothing but what we know about it, i.e., a rotation (of electric charges, of course), then the separation into free magnetic poles is evidently impossible.

It may be useful to point out that, according to the given concept, a magnetic moment, and therefore a spin as well, always corresponds to a real circulating movement and cannot be considered as an intrinsic property without the character of movement. This reemphasizes the fact that static structures in three-dimensional space are not adequate to represent the physical bodies and that the space-time relations are inevitable necessities.

[1] Zocher, H. W. E., and Török, C., *Anais acad. brasil. cienc.*, **20,** 143 (1948).

[2] Wigner, E. P., *Nachr. Ges. Wiss. Goettingen*, **31,** 546 (1932). For more recent literature cf. Luders, G., *Zeits. f. Phys.*, **133,** 325 (1952). cf. also ref 12.

[3] Curie, P., "Oeuvres," Paris, 1908, p. 127.

[4] Circular indicates the asymmetry of axial vectors and paramorphic crystals.

[5] Optical activity is often called a circular double refraction but it would be better labeled as enantiomorphic double refraction, as is easily seen from the comparison of the asymmetry of Faraday effect and optical activity.

[6] Curie, P., *loc. cit.*, p. 133.

[7] Voigt, W., *Lehrbuch der Kristallphysik*, Leipzig, 1928, pp. 261–267.

[8] See, for instance, Bhagavantam, S., and Suryanarayana, D., *Acta Cryst.*, **2,** 24 (1949).

[9] Thomson, W. (Lord Kelvin), *Proc. Roy. Soc. (Edinburgh)*, 123 (1854).

[10] Soret, Ch., *Arch Geneve*, **29,** 4, (1893); **32,** 72, (1894).

[11] Voigt, W., *loc. cit.*, pp. 350, 377.

[12] Onsager, L., *Phys. Rev.*, **37,** 405 (1931).

[13] See Zocher, H., and Török, C., *loc. cit.* It is easily shown that the presence of two higher asymmetries—enantiomorphism, circularity and polarity—along the same direction automatically produces the third. We call the simultaneous presence of the above three asymmetries triasymmetry.

[14] Wooster, W. A., *Crystal Physics*, Cambridge Univ. Press, 1938, p. 7.

[15] Dirac, P. A. M., *Proc. Roy. Soc.*, **A133,** 60 (1931); *Phys. Rev.*, **74,** 817 (1948).

[16] Ehrenhaft, F., *Phys. Rev.* **67,** 207 (1948).

[17] Curie, P., *loc. cit.*, p. 143.

[18] Curie, P., *loc. cit.*, p. 136.

ACTION POTENTIALS FROM AN ARTHROPOD OCELLUS: THE MEDIAN EYE OF LIMULUS*

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During the course of evolution paired lateral eyes have independently developed to a high degree of effectiveness in a number of major groups of bilaterally symmetrical animals. Most of our knowledge of photoreceptors, their fine structure, physiology, biophysics, and biochemistry has been derived from an intensive study of a relatively few examples of such lateral eyes.

TABLE 1

GROUP	MEDIAN PHOTORECEPTOR	OTHER PHOTORECEPTORS
Trilobita[1]	Median eyes Ventral eyes	Compound lateral eyes
Xiphosura[2, 3]	Median eyes Ventral eyes	Compound lateral eyes Rudimentary median and lateral eyes
Eurypterida[1]	Median ocelli	Compound lateral eyes
Pycnogonida[4]	Median ocelli	No lateral eyes
Anachnida[b]	Median ocelli[a] ("Anterior median eyes")	Lateral ocelli[a] (including "posterior median eyes")
Branchiopoda[6]	Naupliar eye	Compound lateral eyes
Ostracoda[6]	Naupliar eye	No lateral eyes in most species
Copepoda[6]	Naupliar eye	No lateral eyes
Cirripedia[6]	Naupliar eye in nauplius larva None in adult	Compound eyes in cypris larva None in adult
Malacostraca[7]	Naupliar eye in larvae and many adults Sixth abdominal ganglion in *Cambarus*[8]	Compound lateral eyes
Insecta[9]	Dorsal ocelli	Compound lateral eyes Lateral ocelli (stemmata) in larvae
Vertebrata[10]	Pineal organ Parapineal organ[b] in fishes, amphibians, and reptiles	Eyes

[a] These retinas are inverted in some groups.

[b] Other parts of the diencephalon may be photosensitive, too.[11]

Concerning median eyes, also widely distributed in several important classes of animals, much less is known. True, their general histological

structure has been extensively examined, particularly in the older literature, but relatively little has been learned either of their functional operation or their sensory significance. Yet in many crustaceans, insects, chelicerates and lower vertebrates median photoreceptor units, quite distinct from the lateral eyes, are known to contribute significantly to the animals' sentience of the environment and its changes (table 1).

Examination of the table indicates that median eyes occur not only as the sole organized photoreceptors of certain animals, like the pycnogonids and most ostracod crustaceans, but more frequently are present along with well-developed lateral compound or camera eyes. It is in fact noteworthy that of the major animal groups with the most highly evolved lateral eyes (crustaceans, pterygote insects, cephalopod mollusks and vertebrates) only in the cephalopods are median photoreceptors completely unknown. Hence to the basic problem of the functional mechanism of the median eye itself must be added the study of the spheres of action and interaction between the median and lateral photoreceptors. Most of the experimental work in this field has been restricted to the latter phase of the general subject although considerable detail is known about the dioptrics of the ocelli of spiders[12, 13] and insects.[14–16] Knowledge of the physiological properties of the median photoreceptors is remarkably incomplete. In no case have the thresholds, visual acuity, spectral sensitivity, critical fusion frequency, intensity discrimination or similar functions been directly measured in simple eyes.

Among the various arthropod groups the reason for this may lie in the minute size of these structures and the general inaccessibility of their nerve supply. Even in a large xiphosuran, 400 mm. in carapace width, the diameter of the median eyes is but 1-2 mm. In many cases, too, the ocelli are located so close to the brain that their nerves are just short stalks difficult of electrode access. Neither of these factors should be a serious drawback, however, in the present state of microelectrode development. The reasons that the photosensitive regions of the diencephalon of the lower vertebrates have not been more closely studied are less obvious. Recently some interesting work[17, 18] on teleosts has been initiated in this neglected field.

Direct electrophysiological analysis of the responses of median photoreceptors has been made in only two cases, both in arthropods. Parry[16] measured the electrical activity in the ocellar nerve of the orthopteran insect, *Locusta migratoria*, and Wulff and Pandazi[19] reported responses in the median eye of *Limulus polyphemus*. No action potentials were observed in either case. For Locusta it was concluded that the ocellar nerve might function normally without conducting propagated impulses; for Limulus the recording technique, which yielded a sort of electroretinogram, was not suitable for detecting action potentials.

From the foregoing it is clear that the study of median photoreceptors presents a striking challenge to the comparative physiologist. The effective integration of morphological, electrophysiological, and ethological data in this field should provide ample rewards to those who attempt it.

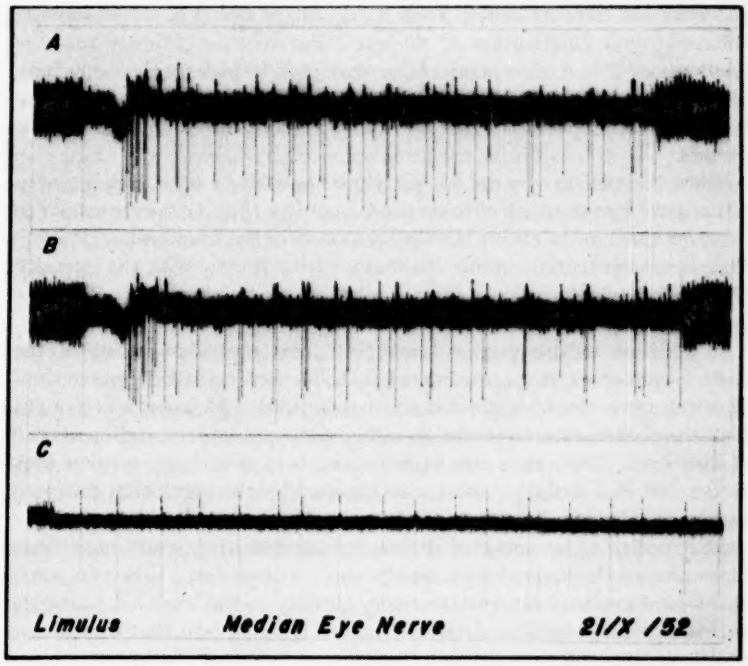

FIGURE 1

Action potentials from the median eye nerve of *Limulus polyphemus* (L.). Records A and B show the responses of a small bundle of fibers to light flashes on the retina. Their duration is indicated by the switching artifacts and the abrupt changes in the noise level of the recording. Record C shows the response of a fine nerve bundle which appears from the electrical record to contain a single active fiber. Both pick-up electrodes were in contact with the nerve; condenser-coupled amplifiers were used. Oscillographic trace photographed on moving paper. The time scale is indicated by the 60-cycle-per-second oscillations in the base line.

The present report describes the first results obtained when the responses of the Limulus median eye are measured in a fashion appropriate for action potential recording. To prepare the eyes a small piece of exoskeleton from the region of the median frontal prosomal spine was dissected in such a way that the two median eyes and their afferent nerves could be excised

normally attached to the cuticle. Curiously, the nerves from the bilaterally symmetrical eyes become fused together and pass asymmetrically to the protocerebrum around one side of the anterior flexure of the digestive tract. A length of a centimeter or more of median eye nerve could be readily secured in this way. The nerve was then split with glass needles into finer and finer strands of fibers until unitary electrical responses were obtained upon illumination of the eye. For recording, fine silver wire electrodes held in a micromanipulator were used to pick up the nerve bundles. Conventional condenser-coupled amplifiers, a cathode-ray oscillograph, and appropriate moving film camera were utilized in obtaining the records.

When the median eyes are adequately stimulated by light such a preparation gives rise to trains of optic nerve impulses (Fig. 1) closely similar to those obtained in the classic Hartline responses of the Limulus lateral eye.[20] Large action potentials, whose frequency varies directly with the intensity of illumination, can be isolated with relative ease as physiologically single units.

In addition to this primary response to external illumination of the median eye cornea, it was discovered that illuminating just the nerve itself sometimes gave rise to conducted action potentials. At first it was thought that these emanated from the so-called endoparietal,[2] or rudimentary,[3] median eye. This organ lies beneath and behind the two median eyes proper and in Limulus† is ordinarily screened by the pigmented carapace and epidermis from light outside the animal. It contributes nerve fibers to the median optic nerve[3] and thus if photosensitive, could have been the source of the anomalous action potentials observed. However, some histological evidence for neurosecretory activity in the lateral eye analogs of this structure has been obtained,[21] and it is not known that they are in addition photoreceptors although they arise embryologically from the ectoderm in close association with the eyes.[22]

More detailed study showed that the peculiar nerve impulses recorded were in fact arising from neither the median eyes nor the endoparietal eye. This was proved by cutting all neuronal connection between these structures and the section of nerve where the impulses were being detected. Exploring this region with a fine pin-point of light revealed that minute (about 0.1 mm. in diameter) darkly pigmented elements scattered along the median eye nerve were the source of these conducted potentials.

So far these structures have not been adequately analyzed histologically. In over-all microscopic appearance they look like small isolated ommatidia of the lateral compound eye. Probably they are the "Zellencomplexe auf der Wanderung" mentioned by Demoll (ref. 3, p. 456). How such elements come to lie within the body imbedded in a sensory nerve, what their functional significance may be, or how systematic their occurrence

is in Xiphosura are questions for future research. The only remotely similar situation occurs in insects. Here the lateral ocelli of the larval stage not infrequently persist as isolated elements buried in the compound lateral eye and its nerve.[23] No measurements have been made of the photosensitivity of these persistent larval organs in insects so even in this respect their parallel to the situation described here in Limulus remains but a crude topographic one.

The demonstration of conducted action potentials in the arthropod median eye is of interest because the only previous serious attempt to find such impulses failed in Locusta.[16] It was concluded that the ocellus in this animal was probably influencing the level of activity in the central nervous system, as it was shown to do, through an electrotonic mechanism. Such spread of potential from the sense organ to the brain, a distance of about 1 mm., was believed to be the means of coordination involved.

Over such a short distance the supposition of this sort of mechanism is not unreasonable although its effectiveness would naturally depend on the resistive and capacitative properties of the neurons involved and the means whereby such a potential would influence post-synaptic elements. A specific electrotonic hypothesis was also proposed independently in the same year to explain inhibition in the vertebrate spinal cord on the basis of an anelectrotonus set up in post-synaptic elements by the short Golgi cells.[24] Recent experiments, however, indicate that such a supposed electrotonic interaction does not provide an adequate explanation in this case.[25]

Further hesitancy about accepting Parry's hypothesis of ocellar function arises from several idiosyncracies of his data for Locusta. As pointed out by Granit,[26] it is most surprising that illumination of the ocellus was found to increase its electrical polarization relative to neighboring regions of the ocellar nerve, whereas darkness depolarized the sense organ. This presents an apparent contradiction to the general rule that stimulation depolarizes excitable tissue of all sorts (e.g., ref. 27). Another curious matter is the presence of synapses between the neurosensory cells of the ocellar retina and the dendrites of the fibers in the ocellar nerve, or tract.[28] These would seem ill suited to an unimpeded electrotonic spread of potential.

Conceivably one might reconcile both of these difficulties with an hypothesis of the following sort. Assume that on illumination current flow originating from depolarized photoreceptor elements produces an anelectrotonus in the peripheral end of the ocellar nerve fibers; assume further that this in turn spreads to and inhibits the pertinent nucleus in the central nervous system. On cessation of illumination the anelectrotonus dies away, and the central inhibition is thereby terminated. Such an hypothesis might be adequate to account for the limited information available for the insect ocellus, but recall that Brock, Coombs, and Eccles[25] on the basis of their data and those of others reject in general the possibility of an electrical

explanation of inhibitory transmission at synapses. In any case it is clear from the present work that the much longer median eye nerve of Limulus conducts nerve impulses of the usual sort carrying coded sensory information to the brain. This report thus constitutes the first record of action potentials from a median eye.

It is clear on the basis of these experiments that the Limulus median eye preparation may provide a useful means of studying the abstruse problem of ocellar function. Many and diverse theories of the functional import of median eyes have been advanced.[29] In most cases the evidence was incomplete and inconclusive. Parry's work on Locusta supports one of the more widely accepted hypotheses of ocellar function in insects, namely that of a stimulatory organ,[30] i.e., one whose excitation enhances reflexes initiated through alternative sensory channels but does not alone evoke specific reflex responses. While in many insects the ocelli thus play only an indirect role in visual responses, in others they take a more direct part.[31]

In Xiphosura, too, the median eyes have been found in preliminary experiments of the author to participate actively in visual orientation. These experiments showed that not only *Limulus polyphemus* (L.) but also *Tachypleus tridentatus* Leach, *Tachypleus gigas* (Müller), and *Carcinoscorpius rotundicauda* (Latreille) all exhibited clear phototactic behavior when both of the lateral compound eyes were covered but the median eyes were normal. Further studies should be undertaken to ascertain whether or not changes in phototactic sign may occur here through the interaction of lateral and median eyes as they have been shown to do in teleosts under the compound influence of the eyes and the pineal body.[17] Similarly the possible involvement of the arthropod median eye in chromatophore and other endocrine control should be investigated. In cyclostomes[32] and teleosts[18] light sensitivity of the pineal region has been clearly implicated in such functions.

Finally, it should be noted that the Limulus median eye preparation provides a good place to investigate the effect of polarized light on simple arthropod eyes. Recent observations on insect larvae[33] and water mites[34] indicate that simple eyes as well as compound eyes in arthropods (for review, see ref. 35) are capable of analyzing polarized light. The Limulus median eye is unusually favorable for this purpose since, as mentioned above, most simple eyes present considerable technical difficulties for electrophysiological study. Experiments are in progress designed to extend the findings on the Limulus lateral eye[36] to the median eye.

In summary, the main points made in the foregoing account are as follows:

1. Review of current knowledge shows that the basic physiological properties and sensory significance of median eyes, occurring widely in both arthropods and lower vertebrates, are largely unknown.

2. In the present work action potentials of single functional elements in the median optic nerve of Limulus have been obtained in response to light stimulation of the median eyes.

3. Small pigmented bodies scattered along the median eye nerve were found to be photosensitive and also to give rise to conducted impulses in the nerve.

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† In juvenile *Tachypleus tridentatus* Leach a transparent window in the carapace and epidermis exposes the "rudimentary" median eye to external illumination (Waterman, unpublished).

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It has been drawn to my attention that a more general inequality than that proved in my paper, "An Inequality with Alternating Signs" (these PROCEEDINGS, **38,** 611–613 (1952)) was given by Professor G. Szegö in a paper entitled "Uber eine Verallgemeinerung des Dirichletschen Integrals" (*Mathematische Zeitschrift* **52,** 683, 1950)).

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Dryden, Hugh Latimer, 1944 (4), National Advisory Committee for Aeronautics, 1724 F Street, N. W., Washington 25, D. C.

DuBois, Eugene Floyd, 1933 (9), Cornell University Medical College, 1300 York Avenue, New York 21, New York

Dubos, René Jules, 1941 (10), Rockefeller Institute for Medical Research 66th Street and York Avenue, New York 21, New York

DuBridge, Lee Alvin, 1943 (3), California Institute of Technology, Pasadena 4, California

Duggar, Benjamin Minge, 1927 (7), Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York

DuMond, Jesse William Monroe, 1953 (3), Department of Physics, California Institute of Technology, Pasadena 4, California

Dunbar, Carl Owen, 1944 (6), Peabody Museum, Yale University, New Haven 11, Connecticut

Dunn, Leslie Clarence, 1943 (8), Columbia University, New York 27, New York

Dunning, John Ray, 1948 (3), 301 Engineering Building, Columbia University, New York 27, New York

Durand, William Frederick, 1917 (4), Mohawk Hotel, 379 Washington Avenue, Brooklyn, New York

du Vigneaud, Vincent, 1944 (9), Department of Biochemistry, Cornell University Medical College, 1300 York Avenue, New York 21, New York

Eckart, Carl, 1953 (13), Scripps Institution of Oceanography, La Jolla, California

Edsall, John Tileston, 1951 (5), Laboratory of Physical Chemistry, Harvard University, 25 Shattuck Street, Boston 15, Massachusetts

Einstein, Albert (1922) 1942† (3), The Institute for Advanced Study, Princeton, New Jersey

Eisenhart, Luther Pfahler, 1922 (1), 25 Alexander Street, Princeton, New Jersey

Elderfield, Robert Cooley, 1949 (5), Department of Chemistry, University of Michigan, Ann Arbor, Michigan

Elvehjem, Conrad Arnold, 1942 (9), Department of Biochemistry, University of Wisconsin, Madison 6, Wisconsin

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Emerson, Robert, 1953 (7), Department of Botany, University of Illinois, Urbana, Illinois

Enders, John Franklin, 1953 (10), The Children's Hospital, 300 Longwood Avenue, Boston 15, Massachusetts

Epstein, Paul Sophus, 1930 (3), 1484 Oakdale Street, Pasadena 4, California

Erlanger, Joseph, 1922 (9), 660 South Kingshighway, St. Louis 10, Missouri

Evans, Griffith Conrad, 1933 (1), Department of Mathematics, University of California, Berkeley 4, California

Evans, Herbert McLean, 1927 (9), Institute of Experimental Biology, University of California, Berkeley 4, California

Ewing, William **Maurice,** 1948 (13), Columbia University, Lamont Geological Observatory, Torrey Cliff, Palisades, New York

Eyring, Henry, 1945 (5), Graduate School, University of Utah, Salt Lake City, Utah

Fenn, Wallace Osgood, 1943 (9), School of Medicine and Dentistry, University of Rochester, 260 Crittenden Boulevard, Rochester 7, New York

Fermi, Enrico, 1945 (3), Institute for Nuclear Studies, University of Chicago, Chicago 37, Illinois

Fieser, Louis Frederick, 1940 (5), Harvard University, Cambridge 38, Massachusetts

Fleming, John Adam, 1938 (13), Carnegie Institution of Washington, 1530 P Street, N. W., Washington 5, D. C.

Fletcher, Harvey, 1935 (4), Engineering Sciences Department, Brigham Young University, Provo, Utah

Flory, Paul John, 1953 (5), Department of Chemistry, Cornell University, Ithaca, New York

Folkers, Karl August, 1948 (5), Organic and Biological Chemical Research Division, Merck & Co., Inc., Rahway, New Jersey

Foote, Paul Darwin, 1943 (4), Gulf Research and Development Company, P. O. Drawer 2038, Pittsburgh 30, Pennsylvania

Forbes, Alexander, 1936 (9), Harland Street, Milton, Massachusetts

Francis, Thomas, Jr., 1948 (10), Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Michigan

Franck, James, 1944 (3), Institute of Radiobiology and Biophysics, University of Chicago, Chicago 37, Illinois

Fred, Edwin Broun, 1931 (7), University of Wisconsin, Madison 6, Wisconsin

Fruton, Joseph Stewart, 1952 (9), Department of Physiological Chemistry, Yale University School of Medicine, 333 Cedar Street, New Haven 11, Connecticut

Fuoss, Raymond Matthew, 1951 (5), Sterling Chemistry Laboratory, Yale University, 225 Prospect Street, New Haven 11, Connecticut

Fuson, Reynold Clayton, 1944 (5), 263 Noyes Laboratory, University of Illinois, Urbana, Illinois

Gamble, James Lawder, 1945 (10), 33 Edge Hill Road, Brookline, Massachusetts

Gamow, George, 1953 (3), Department of Physics, George Washington University, Washington, D. C.

Gasser, Herbert Spencer, 1934 (9), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, New York

Gesell, Arnold, 1947 (12), Gesell Institute of Child Development, 310 Prospect Street, New Haven 11, Connecticut

Giauque, William Francis, 1936 (5), University of California, Berkeley 4, California

Gibbs, William Francis, 1949 (4), One Broadway, New York, New York

Gilliland, Edwin Richard, 1948 (4), Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge 39, Massachusetts

Gilluly, James, 1947 (6), United States Geological Survey, Denver Federal Center, Denver 14, Colorado

Gilman, Henry, 1945 (5), Department of Chemistry, Iowa State College, Ames, Iowa

Goddard, David Rockwell, 1950 (7), Botanical Laboratory, University of Pennsylvania, Philadelphia 4, Pennsylvania

Goldschmidt, Richard Benedikt, 1947 (8), Department of Zoology, University of California, Berkeley 4, California

Goodpasture, Ernest William, 1937 (10), Vanderbilt University, Nashville 4, Tennessee

Goudsmit, Samuel Abraham, 1947 (3), Department of Physics, Brookhaven National Laboratory, Upton, Long Island, New York

Graham, Clarence Henry, 1946 (12), Department of Psychology, Columbia University, New York 27, New York

Graham, Evarts Ambrose, 1941 (10), Barnes Hospital, 600 South Kingshighway, St. Louis 10, Missouri

Greenewalt, Crawford Hallock, 1952 (4), E. I. du Pont de Nemours and Company, Incorporated, Wilmington, Delaware

Gregory, William King, 1927 (6), American Museum of Natural History, 77th Street and Central Park West, New York 24, New York

Griggs, David Tressel, 1952 (13), Institute of Geophysics, University of California, Los Angeles 24, California

Gunn, Ross, 1951 (13), United States Weather Bureau, Department of Commerce, Washington 25, D. C.

Gutenberg, Beno, 1945 (13), Seismological Laboratory, 220 North San Rafael Avenue, Pasadena 2, California

Hamburger, Viktor, 1953 (8), Department of Zoology, Washington University, St. Louis 5, Missouri

Hammett, Louis Plack, 1943 (5), Department of Chemistry, Columbia University, New York 27, New York

Harlow, Harry F., 1951 (12), Department of Psychology, University of Wisconsin, Madison 6, Wisconsin

Harned, Herbert Spencer, 1950 (5), Sterling Chemistry Laboratory, Yale University, 225 Prospect Street, New Haven 11, Connecticut

Harrison, Ross Granville, 1913 (8), Osborn Zoological Laboratory, Yale University, New Haven 11, Connecticut

Hartline, Haldan Keffer, 1948 (9), Department of Biophysics, Johns Hopkins University, Baltimore 18, Maryland

Hartman, Carl Gottfried, 1937 (8), Ortho Research Foundation, Raritan, New Jersey

Harvey, Edmund Newton, 1934 (8), Guyot Hall, Princeton University, Princeton, New Jersey

Hastings, Albert Baird, 1939 (9), Department of Biological Chemistry, Harvard Medical School, 25 Shattuck Street, Boston 15, Massachusetts

Heidelberger, Michael, 1942 (10), College of Physicians and Surgeons, 620 West 168th Street, New York 32, New York

Hendricks, Sterling Brown, 1952 (5), Plant Industry Station, Beltsville, Maryland

Herrick, Charles Judson, 1918 (8), 236 Morningside Drive, S. E., Grand Rapids 6, Michigan

Hess, Harry Hammond, 1952 (6), Department of Geology, Princeton University, Princeton, New Jersey

Hewett, Donnel Foster, 1937 (6), 1460 Rose Villa Street, Pasadena 4, California

Hildebrand, Joel Henry, 1929 (5), Department of Chemistry, University of California, Berkeley 4, California

Hilgard, Ernest Ropiequet, 1948 (12), Graduate Division, Stanford University, Stanford, California

Hille, Carl Einar, 1953 (1), Department of Mathematics, Yale University, New Haven 11, Connecticut

Hirschfelder, Joseph Oakland, 1953 (5), University of Wisconsin, P. O. Box 2127, Madison 6, Wisconsin

Hisaw, Frederick Lee, 1947 (8), The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge 38, Massachusetts

Hooton, Earnest Albert, 1935 (11), Peabody Museum, Harvard University, Cambridge 38, Massachusetts

Hoover, Herbert Clark, 1922 (4), Waldorf Astoria Towers, New York, New York

Horsfall, Frank Lappin, Jr., 1948 (10), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, New York

Horsfall, James Gordon, 1953 (7), Connecticut Agricultural Experiment Station, New Haven 4, Connecticut

Houston, William Vermillion, 1943 (3), The Rice Institute, Houston, Texas

Hubble, Edwin Powell, 1927 (2), Mount Wilson Observatory, Pasadena 4, California

Hubbs, Carl Leavitt, 1952 (8), Scripps Institution of Oceanography, La Jolla, California

Huggins, Charles Brenton, 1949 (10), Department of Surgery, University of Chicago, 950 East 59th Street, Chicago 37, Illinois

Hull, Albert Wallace, 1929 (3), General Electric Research Laboratory, The Knolls, Schenectady, New York

Hunsaker, Jerome Clark, 1935 (4), Massachusetts Institute of Technology, Cambridge 39, Massachusetts

Hunter, Walter Samuel, 1935 (12), Brown University, Providence 12, Rhode Island

Hutchinson, George Evelyn, 1950 (8), Osborn Zoological Laboratory, Yale University, New Haven 11, Connecticut

Irwin, Malcolm Robert, 1950 (8), Department of Genetics, University of Wisconsin, Madison 6, Wisconsin

Iselin, Columbus O'Donnell, 1951 (13), Woods Hole Oceanographic Institution, Woods Hole, Massachusetts

Ives, Herbert Eugene, 1933 (3), 32 Laurel Place, Upper Montclair, New Jersey

Jacobs, Merkel Henry, 1939 (8), School of Medicine, University of Pennsylvania, Philadelphia 4, Pennsylvania

Jacobs, Walter Abraham, 1932 (5), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, New York

Jeffries, Zay, 1939 (4), General Electric Company, 1 Plastics Avenue, Pittsfield, Massachusetts

Johnson, John Raven, 1948 (5), Department of Chemistry, Cornell University, Ithaca, New York

Johnson, William Summer, 1952 (5), Department of Chemistry, University of Wisconsin, Madison 6, Wisconsin

Jones, Donald Forsha, 1939 (7), Department of Genetics, Connecticut Agricultural Experiment Station, P. O. Box 1106, New Haven 4, Connecticut

Joy, Alfred Harrison, 1944 (2), Mount Wilson Observatory, Pasadena 4, California

Kasner, Edward, 1917 (1), 430 West 116th Street, New York 27, New York

Kaufmann, Berwind Petersen, 1952 (7), Department of Genetics, Carnegie Institution of Washington, Cold Spring Harbor, Long Island, New York

Kelley, Walter Pearson, 1943 (6), 120 Hilgard Hall, University of California, Berkeley 4, California

Kellogg, Arthur Remington, 1951 (8), United States National Museum, Smithsonian Institution, Washington 25, D. C.

Kelly, Mervin J., 1945 (4), Bell Telephone Laboratories, 463 West Street, New York 14, New York

Kemble, Edwin Crawford, 1931 (3), Physics Laboratories, Harvard University, Cambridge 38, Massachusetts

Kendall, Edward Calvin, 1950 (9), 3 Queenston Place, Princeton, New Jersey

Kent, Robert Harrington, 1951 (3), Ballistic Research Laboratories, Aberdeen Proving Ground, Maryland

Kerst, Donald William, 1951 (3), Department of Physics, University of Illinois, Urbana, Illinois

Kettering, Charles Franklin, 1928 (4), General Motors Corporation, Detroit 2, Michigan

Keyes, Frederick George, 1930 (5), Massachusetts Institute of Technology, Cambridge 39, Massachusetts

Kharasch, Morris Selig, 1946 (5), Department of Chemistry, University of Chicago, Chicago 37, Illinois

Kidder, Alfred Vincent, 1936 (11), 41 Holden Street, Cambridge 38, Massachusetts

King, Arthur Scott, 1941 (2), Mount Wilson Observatory, Pasadena 4, California

King, Charles Glen, 1951 (9), The Nutrition Foundation, Incorporated, Chrysler Building, New York 17, New York

Kirkwood, John Gamble, 1942 (5), Sterling Chemistry Laboratory, Yale University, 225 Prospect Street, New Haven 11, Connecticut

Kistiakowsky, George Bogdan, 1939 (5), Department of Chemistry, Harvard University, 12 Oxford Street, Cambridge 38, Massachusetts

Kluckhohn, Clyde Kay Maben, 1952 (11), Russian Research Center, Harvard University, Cambridge 38, Massachusetts

Knopf, Adolph, 1931 (6), Department of Geology, Stanford University, Stanford, California

Köhler, Wolfgang, 1947 (12), Swarthmore College, Swarthmore, Pennsylvania

Kraus, Charles August, 1925 (5), Brown University, Providence 12, Rhode Island

Kroeber, Alfred L., 1928 (11), Department of Anthropology, University of California, Berkeley 4, California

Kuiper, Gerard Peter, 1950 (2), Yerkes Observatory, University of Chicago, Williams Bay, Wisconsin

Kunkel, Louis Otto, 1932 (7), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, New York

Lambert, Walter Davis, 1949 (13), P. O. Box 687, Canaan, Connecticut

LaMer, Victor Kuhn, 1945 (5), Department of Chemistry, Columbia University, New York 27, New York

Land, Edwin Herbert, 1953 (3), Polaroid Corporation, Cambridge 39, Massachusetts

Langmuir, Irving, 1918 (5), Research Laboratory, General Electric Company, P. O. Box 1088, Schenectady, New York

Larsen, Esper S., Jr., 1944 (6), Apt. 502 H, 3930 Connecticut Avenue, N. W., Washington 8, D. C.

Lashley, Karl Spencer, 1930 (12), Yerkes Laboratories of Primate Biology, Incorporated, Orange Park, Florida

Latimer, Wendell Mitchell, 1940 (5), Gilman Hall, University of California, Berkeley 4, California

Lauritsen, Charles Christian, 1941 (3), California Institute of Technology, Pasadena 4, California

Lawrence, Ernest Orlando, 1934 (3), Radiation Laboratory, University of California, Berkeley 4, California

Lefschetz, Solomon, 1925 (1), Fine Hall, Princeton University, 129 Broadmead Street, Princeton, New Jersey

Leith, Charles Kenneth, 1920 (6), Sheraton Park Hotel, Washington 8, D. C.

Lewis, Howard Bishop, 1949 (9), Department of Biological Chemistry, University of Michigan, Ann Arbor, Michigan

Lewis, Warren Harmon, 1936 (8), The Wistar Institute of Anatomy and Biology, Philadelphia 4, Pennsylvania

Lewis, Warren Kendall, 1938 (4), Massachusetts Institute of Technology, Cambridge 39, Massachusetts

Libby, Willard Frank, 1950 (5), Institute for Nuclear Studies, University of Chicago, Chicago 37, Illinois

Lind, Samuel Colville, 1930 (5), P. O. Box P, Oak Ridge, Tennessee

Lindsley, Donald Benjamin, 1952 (12), Department of Psychology, University of California, Los Angeles 24, California

Link, Karl Paul, 1946 (9), Department of Biochemistry, University of Wisconsin, Madison 6, Wisconsin

Linton, Ralph, 1945 (11), Institute of Human Relations, Yale University, 333 Cedar Street, New Haven 11, Connecticut

Lipmann, Fritz Albert, 1950 (9), Biochemical Research Laboratory, Massachusetts General Hospital, Boston 14, Massachusetts

Little, Clarence Cook, 1945 (10), Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine

Lloyd, David Pierce Caradoc, 1953 (9), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, New York

Loeb, Leo, 1937 (10), 40 Crestwood Drive, St. Louis 5, Missouri

Loeb, Robert Frederick, 1946 (9), College of Physicians and Surgeons, 620 West 168th Street, New York 32, New York

Long, Cyril Norman Hugh, 1948 (9), Yale University School of Medicine, 333 Cedar Street, New Haven 11, Connecticut

Long Esmond Ray, 1946 (10), Henry Phipps Institute, 7th and Lombard Streets, Philadelphia 47, Pennsylvania

Longsworth, Lewis Gibson, 1947 (5), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, New York

Longwell, Chester Ray, 1935 (6), Kirtland Hall, Yale University, New Haven 11, Connecticut

Loomis, Alfred Lee, 1941 (4), The Loomis Laboratory, Room 2420, 14 Wall Street, New York 5, New York

Loomis, Francis Wheeler, 1949 (3), 804 West Illinois Street, Urbana, Illinois

Lorente de Nó, Rafael, 1950 (9), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, New York

Lothrop, Samuel Kirkland, 1951 (11), Peabody Museum, Harvard University, Cambridge 38, Massachusetts

Lovering, Thomas Seward, 1949 (6), 8001 West 17th Avenue, Lakewood, Colorado

Lowie, Robert Harry, 1931 (11), University of California, Berkeley 4, California

Lyman, Theodore, 1917 (3), Research Laboratory of Physics, Harvard University, Cambridge 38, Massachusetts

Macelwane, James Bernard, **S.J.,** 1944 (13), 3621 Olive Street, St. Louis 8, Missouri

MacInnes, Duncan Arthur, 1937 (5), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, New York

Mac Lane, Saunders, 1949 (1), Department of Mathematics, University of Chicago, Chicago 37, Illinois

Mangelsdorf, Paul Christoph, 1945 (7), Botanical Museum, Harvard University, Cambridge 38, Massachusetts

Mann, Frank Charles, 1950 (9), Mayo Foundation, University of Minnesota, Rochester, Minnesota

Marshall, Eli Kennerly, **Jr.,** 1943 (9), School of Medicine, Johns Hopkins University, 710 North Washington Street, Baltimore 5, Maryland

Marvel, Carl Shipp, 1938 (5), Department of Chemistry, University of Illinois, Urbana, Illinois

Mason, Max, 1923 (3), 1035 Harvard Avenue, Claremont, California

Maxcy, Kenneth Fuller, 1950 (10), School of Hygiene and Public Health, Johns Hopkins University, 615 North Wolfe Street, Baltimore 5, Maryland

Mayall, Nicholas Ulrich, 1949 (2), Lick Observatory, Mount Hamilton, California

Mayer, Joseph Edward, 1946 (5), Institute for Nuclear Studies, University of Chicago, Chicago 37, Illinois

Maynard, Leonard Amby, 1944 (9), School of Nutrition, Cornell University, Ithaca, New York

McClintock, Barbara, 1944 (7), Carnegie Institution of Washington, Cold Spring Harbor, Long Island, New York

McCollum, Elmer Verner, 1920 (9), Gilman Hall, Johns Hopkins University, Baltimore 18, Maryland

McElvain, Samuel Marion, 1949 (5), Department of Chemistry, University of Wisconsin, Madison 6, Wisconsin

McMaster, Philip Duryeé, 1952 (10), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, New York

McMillan, Edwin Mattison, 1947 (3), Radiation Laboratory, University of California, Berkeley 4, California

McShane, Edward James, 1948 (1), Department of Mathematics, University of Virginia, Charlottesville, Virginia

Mead, Warren Judson, 1939 (6), 88 Rutledge Road, Belmont 78, Massachusetts

Meek, Walter Joseph, 1947 (9), Department of Physiology, University of Wisconsin, Madison 6, Wisconsin

Mees, Charles Edward **Kenneth,** 1950 (5), Eastman Kodak Company, Kodak Park Works, Rochester 4, New York

Mendenhall, Walter Curran, 1932 (6), 9 East Lenox Street, Chevy Chase 15, Maryland

Menzel, Donald Howard, 1948 (2), Harvard College Observatory, Cambridge 38, Massachusetts

Merica, Paul Dyer, 1942 (4), 67 Wall Street, New York 5, New York

Merrill, Paul Willard, 1929 (2), 813 Santa Barbara Street, Pasadena 4, California

Metz, Charles William, 1948 (8), Department of Zoology, University of Pennsylvania, Philadelphia 4, Pennsylvania

Meyer, Karl Friederich, 1940 (10), George Williams Hooper Foundation, University of California Medical Center, San Francisco 22, California

Miles, Walter Richard, 1933 (12), Yale University School of Medicine, 333 Cedar Street, New Haven 11, Connecticut

Millikan, Robert Andrews, 1915 (3), California Institute of Technology, Pasadena 4, California

Mitchell, Samuel Alfred, 1933 (2), Leander McCormick Observatory, University Station, Charlottesville, Virginia

Moore, Carl Richard, 1944 (8), University of Chicago, Chicago 37, Illinois

Moore, Robert Lee, 1931 (1), University of Texas, Austin 12, Texas

Morse, Harold Marston, 1932 (1), The Institute for Advanced Study, Princeton, New Jersey

Mueller, John Howard, 1945 (10), Harvard Medical School, 25 Shattuck Street, Boston 15, Massachusetts

Muller, Hermann Joseph, 1931 (8), 101 Science Hall, Indiana University, Bloomington, Indiana. June 1, 1953 to September 1, 1954: 5203 Pilikai Street, Honolulu, T. H.

Mulliken, Robert Sanderson, 1936 (3), Laboratory of Molecular Structure and Spectra, University of Chicago, Chicago 37, Illinois

Murnaghan, Francis Dominic, 1942 (1), Instituto Tecnologico de Aeronautica, São Jose dos Campos, Estado de São Paulo, Brasil

Murphree, Eger Vaughan, 1950 (4), Standard Oil Development Company, 15 West 51st Street, New York 19, New York

Nicholas, John Spangler, 1949 (8), Osborn Zoological Laboratory, Yale University, New Haven 11, Connecticut

Nicholson, Seth Barnes, 1937 (2), Mount Wilson Observatory, Pasadena 4, California

Niemann, Carl George, 1952 (5), Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena 4, California

Nier, Alfred Otto C., 1950 (3), Department of Physics, University of Minnesota, Minneapolis 14, Minnesota

Nissen, Henry W., 1953 (12), Yerkes Laboratories of Primate Biology, Incorporated, Orange Park, Florida

Nolan, Thomas Brennan, 1951 (6), United States Geological Survey, Department of the Interior, Washington 25, D. C.

Northrop, John Howard, 1934 (9), Department of Bacteriology, University of California, Berkeley 4, California

Noyes, William Albert, Jr., 1943 (5), Department of Chemistry, University of Rochester, Rochester 3, New York

Oncley, John Lawrence, 1947 (9), Laboratory of Physical Chemistry, Harvard University, 25 Shattuck Street, Boston 15, Massachusetts

Onsager, Lars, 1947 (5), Sterling Chemistry Laboratory, Yale University, 225 Prospect Street, New Haven 11, Connecticut

Opie, Eugene Lindsay, 1923 (10), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, New York

Oppenheimer, J. Robert, 1941 (3), The Institute for Advanced Study, Princeton, New Jersey

Osterhout, Winthrop John Vanleuven, 1919 (7), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, New York

Painter, Theophilus Shickel, 1938 (8), University of Texas, Austin 12, Texas

Patterson, John Thomas, 1941 (8), Department of Zoology, University of Texas, Austin 12, Texas

Paul, John Rodman, 1945 (10), Yale University School of Medicine, 333 Cedar Street, New Haven 11, Connecticut

Pauling, Linus, 1933 (5), Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena 4, California

Pegram, George Braxton, 1949 (3), Columbia University, New York 27, New York

Pekeris, Chaim Leib, 1952 (13), Department of Applied Mathematics, The Weizmann Institute of Science, Rehovot, Israel

Peters, John Punnett, 1947 (9), Department of Internal Medicine, Yale University School of Medicine, 333 Cedar Street, New Haven 11, Connecticut

Pierce, George Washington, 1920 (3), Cruft Laboratory, Harvard University, Cambridge 38, Massachusetts

Piggot, Charles Snowden, 1946 (13), 2200 Decatur Place, N. W., Washington 8, D. C.

Pillsbury, Walter Bowers, 1925 (12), Haven Hall, University of Michigan, Ann Arbor, Michigan

Pitzer, Kenneth Sanborn, 1949 (5), Department of Chemistry and Chemical Engineering, University of California, Berkeley 4, California

Purcell, Edward Mills, 1951 (3), Lyman Laboratory of Physics, Harvard University, Cambridge 38, Massachusetts

Rabi, Isidor Isaac, 1940 (3), Department of Physics, Columbia University, New York 27, New York

Ramsey, Norman Foster, 1952 (3), Lyman Laboratory of Physics, Harvard University, Cambridge 38, Massachusetts

Raper, Kenneth Bryan, 1949 (7), Department of Bacteriology, University of Wisconsin, Madison 6, Wisconsin

Raymond, Arthur Emmons, 1950 (4), Douglas Aircraft Company, Incorporated, 3000 Ocean Park Boulevard, Santa Monica, California

Reeside, John Bernard, **Jr.,** 1945 (6), Box 177, Hyattsville, Maryland

Reichelderfer, Francis Wilton, 1945 (13), United States Weather Bureau, Department of Commerce, Washington 25, D. C.

Rhoades, Marcus Morton, 1946 (7), Department of Botany, University of Illinois, Urbana, Illinois

Richards, Alfred Newton, 1927 (9), School of Medicine, University of Pennsylvania, Philadelphia 4, Pennsylvania

Richter, Curt Paul, 1948 (12), Johns Hopkins Hospital, Baltimore 5, Maryland

Riddle, Oscar, 1939 (8), Route 4, Plant City, Florida

Riker, Albert Joyce, 1951 (7), Department of Plant Pathology, University of Wisconsin, Madison 6, Wisconsin

Rittenberg, David, 1953 (9), College of Physicians and Surgeons, 630 West 168th Street, New York 32, New York

Rivers, Thomas Milton, 1934 (10), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, New York

Robbins, William Jacob, 1940 (7), New York Botanical Garden, Bronx Park (Fordham Station), New York 58, New York

Robertson, Howard Percy, 1951 (3), Norman Bridge Laboratory of Physics, California Institute of Technology, Pasadena 4, California

Robertson, Oswald Hope, 1943 (10), Department of Biology, Stanford University, Stanford, California

Rodebush, Worth Huff, 1938 (5), University of Illinois, Urbana, Illinois

Romer, Alfred Sherwood, 1944 (8), Museum of Comparative Zoology at Harvard College, Oxford Street, Cambridge 38, Massachusetts

Rose, William Cumming, 1936 (9), University of Illinois, Urbana, Illinois

Ross, Frank Elmore, 1930 (2), 1100 Mt. Lowe Drive, Altadena, California

Rossby, Carl-Gustaf Arvid, 1943 (13), Institute of Meteorology, University of Stockholm, Stockholm, Sweden

Rossi, Bruno Benedetto, 1950 (3), Department of Physics, Massachusetts Institute of Technology, Cambridge 39, Massachusetts

Rossini, Frederick Dominic, 1951 (5), Department of Chemistry, Carnegie Institute of Technology, Pittsburgh 13, Pennsylvania

Rous, Francis Peyton, 1927 (10), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, New York

Rubey, William Walden, 1945 (6), United States Geological Survey, Department of the Interior, Washington 25, D. C.

Ruedemann, Rudolf, 1928 (6), 161 Dana Avenue, Albany 3, New York

Russell, Henry Norris, 1918 (2), Princeton University, Princeton, New Jersey

Sabin, Albert Bruce, 1951 (10), The Children's Hospital Research Foundation, Elland Avenue and Bethesda, Cincinnati 29, Ohio

Sabin, Florence Rena, 1925 (10), 1333 East 10th Avenue, Denver 3, Colorado

Saunders, Frederick Albert, 1925 (3), South Hadley, Massachusetts

Savage, John Lucian, 1949 (4), 1651 Dahlia Street, Denver 7, Colorado

Sax, Karl, 1941 (7), Harvard University, Cambridge 38, Massachusetts

Scatchard, George, 1946 (5), Department of Chemistry, Massachusetts Institute of Technology, Cambridge 39, Massachusetts

Schairer, John Frank, 1953 (6), Geophysical Laboratory, Carnegie Institution of Washington, 2801 Upton Street, N. W., Washington 8, D. C.

Schlesinger, Hermann Irving, 1948 (5), Department of Chemistry, University of Chicago, Chicago 37, Illinois

Schmidt, Carl Frederic, 1949 (9), Laboratory of Pharmacology, School of Medicine, University of Pennsylvania, Philadelphia 4, Pennsylvania

Schmitt, Francis Otto, 1948 (8), Department of Biology, Massachusetts Institute of Technology, Cambridge 39, Massachusetts

Schrader, Franz, 1951 (8), Department of Zoology, Columbia University, New York 27, New York

Schultz, Adolph Hans, 1939 (11), Anthropologisches Institut, Sempersteig 3, Zurich, Switzerland

Schwinger, Julian, 1949 (3), Department of Physics, Harvard University, Cambridge 38, Massachusetts

Seaborg, Glenn Theodore, 1948 (5), Department of Chemistry, University of California, Berkeley 4, California

Seares, Frederick Hanley, 1919 (2), 351 Palmetto Drive, Pasadena 2, California

Segrè, Emilio, 1952 (3), Department of Physics, University of California, Berkeley 4, California

Seitz, Frederick, 1951 (3), Department of Physics, University of Illinois, Urbana, Illinois

Serber, Robert, 1952 (3), Department of Physics, Columbia University, New York 27, New York

Shaffer, Philip Anderson, 1928 (9), School of Medicine, Washington University, Euclid Avenue and Kingshighway, St. Louis 10, Missouri

Shapiro, Harry Lionel, 1949 (11), American Museum of Natural History, Central Park West at 79th Street, New York 24, New York

Shapley, Harlow, 1924 (2), Harvard College Observatory, Cambridge 38, Massachusetts

Shedlovsky, Theodore, 1953 (5), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, New York

Sherman, Henry Clapp, 1933 (9), Columbia University, New York 27, New York

Shockley, William, 1951 (4), Bell Telephone Laboratories, Murray Hill, New Jersey

Shope, Richard Edwin, 1940 (10), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, New York

Simpson, George Gaylord, 1941 (6), American Museum of Natural History, 77th Street and Central Park West, New York 24, New York

Sinnott, Edmund Ware, 1936 (7), Hall of Graduate Studies, Yale University, New Haven 11, Connecticut

Skinner, Burrhus Frederic, 1950 (12), Memorial Hall, Harvard University, Cambridge 38, Massachusetts

Slater, John Clarke, 1932 (3), Massachusetts Institute of Technology, Cambridge 39, Massachusetts

Slepian, Joseph, 1941 (4), Westinghouse Electric Corporation, East Pittsburgh, Pennsylvania

Slichter, Louis Byrne, 1944 (13), Institute of Geophysics, University of California, Los Angeles 24, California

Slipher, Vesto Melvin, 1921 (2), Lowell Observatory, Flagstaff, Arizona

Small, Lyndon Frederick, 1941 (5), National Institutes of Health, Bethesda 14, Maryland

Smith, Gilbert Morgan, 1948 (7), Department of Biology, Stanford University, Stanford, California

Smith, Homer William, 1945 (9), 477 First Avenue, New York 16, New York

Smith, Lee Irvin, 1944 (5), School of Chemistry, University of Minnesota, Minneapolis 14, Minnesota

Smith, Paul Althaus, 1947 (1), Department of Mathematics, Columbia University, New York 27, New York

Smith, Philip Edward, 1939 (8), College of Physicians and Surgeons, 630 West 168th Street, New York 32, New York

Soderberg, Carl Richard, 1947 (4), Massachusetts Institute of Technology, Cambridge 39, Massachusetts

Sonneborn, Tracy Morton, 1946 (8), 109 Wylie Hall, Indiana University, Bloomington, Indiana

Spedding, Frank Harold, 1952 (5), Box 14-A, Station A, Institute for Atomic Research, Iowa State College, Ames, Iowa

Spier, Leslie, 1946 (11), P. O. Box 880, Santa Cruz, California. February 1 to June 1, annually: University of New Mexico, Albuquerque, New Mexico

Spitzer, Lyman, Jr., 1952 (2), Princeton University Observatory, 14 Prospect Avenue, Princeton, New Jersey

Stadie, William Christopher, 1945 (9), 821 Maloney Clinic, 36th and Spruce Streets, Philadelphia 4, Pennsylvania

Stadler, Lewis John, 1938 (7), University of Missouri, Columbia, Missouri

Stakman, Elvin Charles, 1934 (7), University Farm, St. Paul 1, Minnesota

Stanley, Wendell Meredith, 1941 (9), Biochemistry and Virus Laboratory, University of California, Berkeley 4, California

Stebbins, George Ledyard, Jr., 1952 (7), Division of Genetics, College of Agriculture, University of California, Davis, California

Stebbins, Joel, 1920 (2), Lick Observatory, Mt. Hamilton, California

Stern, Curt, 1948 (8), Department of Zoology, University of California, Berkeley 4, California

Stern, Otto, 1945 (3), 759 Cragmont Avenue, Berkeley 8, California

Stevens, Stanley Smith, 1946 (12), Department of Psychology, Harvard University, Cambridge 38, Massachusetts

Stewart, George Walter, 1938 (3), Hall of Physics, State University of Iowa, Iowa City, Iowa

Stone, Calvin Perry, 1943 (12), Stanford University, Stanford, California

Stone, Marshall Harvey, 1938 (1), 303 Eckhart Hall, University of Chicago, Chicago 37, Illinois

Stratton, Julius Adams, 1950 (4), Massachusetts Institute of Technology, Cambridge 39, Massachusetts

Street, Jabez Curry, 1953 (3), Jefferson Physical Laboratory, Harvard University, Cambridge 38, Massachusetts

Struve, Otto, 1937 (2), Department of Astronomy, University of California, Berkeley 4, California

Sturtevant, Alfred Henry, 1930 (8), California Institute of Technology, Pasadena 4, California

Suits, Chauncey Guy, 1946 (4), General Electric Research Laboratory, The Knolls, Schenectady, New York

Sumner, James Batcheller, 1948 (9), Savage Hall, New York State College of Agriculture, Cornell University, Ithaca, New York

Taliaferro, William Hay, 1940 (8), University of Chicago, 5724 Ellis Avenue, Chicago 37, Illinois

Tatum, Edward Lawrie, 1952 (9), Department of Biological Sciences, Stanford University, Stanford, California

Teller, Edward, 1948 (3), Department of Physics, University of California, Berkeley 4, California

Terman, Frederick Emmons, 1946 (4), School of Engineering, Stanford University, Stanford, California

Terman, Lewis Madison, 1928 (12), 761 Dolores Street, Stanford University, Stanford, California

Thimann, Kenneth Vivian, 1948 (7), The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge 38, Massachusetts

Thom, Charles, 1937 (7), 207 Grant Street, Port Jefferson, New York

Thomas, Charles Allen, 1948 (4), Monsanto Chemical Company, 1700 South Second Street, St. Louis 4, Missouri

Thomas, Tracy Yerkes, 1941 (1), Swain Hall, Indiana University, Bloomington, Indiana

Thompson, Thomas Gordon, 1951 (13), Oceanographic Laboratories, University of Washington, Seattle 5, Washington

Thurstone, Louis Leon, 1938 (12), Nash Hall, University of North Carolina, Chapel Hill, North Carolina

Tillett, William Smith, 1951 (10), New York University College of Medicine, 477 First Avenue, New York 16, New York

Timoshenko, Stephen Prokop, 1940 (4), Room 262, Engineering Building, Stanford University, Stanford, California

Tishler, Max, 1953 (5), Process Research and Development Division, Merck & Co., Inc., Rahway, New Jersey

Tolman, Edward Chace, 1937 (12), Department of Psychology, University of California, Berkeley 4, California

Tozzer, Alfred Marston, 1942 (11), Peabody Museum, Harvard University, Cambridge, 38, Massachusetts

Trumpler, Robert Julius, 1932 (2), Route 2, Box 1750, Aptos, California

Tuve, Merle Antony, 1946 (3), Department of Terrestrial Magnetism, Carnegie Institution of Washington, 5241 Broad Branch Road, N. W., Washington 15, D. C.

Twitty, Victor Chandler, 1950 (8), Department of Biological Sciences, Stanford University, Stanford, California

Tyzzer, Ernest Edward, 1942 (10), 175 Water Street, Wakefield, Massachusetts

Urey, Harold Clayton, 1935 (5), Institute for Nuclear Studies, University of Chicago, Chicago 37, Illinois

Van Niel, Cornelis Bernardus, 1945 (7), Hopkins Marine Station of Stanford University, Pacific Grove, California

Van Slyke, Donald Dexter, 1921 (9), Brookhaven National Laboratory, Upton, Long Island, New York

Van Vleck, John Hasbrouck, 1935 (3), Lyman Laboratory of Physics, Harvard University, Cambridge 38, Massachusetts

Veblen, Oswald, 1919 (1), The Institute for Advanced Study, Princeton, New Jersey

Vickery, Hubert Bradford, 1943 (9), Connecticut Agricultural Experiment Station, New Haven 4, Connecticut

von Kármán, Theodore, 1938 (4), 1501 South Marengo Avenue, Pasadena California

von Neumann, John, 1937 (1), The Institute for Advanced Study, Princeton, New Jersey

Waksman, Selman Abraham, 1942 (7), Institute of Microbiology, Rutgers University, New Brunswick, New Jersey

Wald, George, 1950 (9), The Biological Laboratories, Harvard University, 16 Divinity Avenue, Cambridge 38, Massachusetts

Walker, John Charles, 1945 (7), 206 Horticulture Building, University of Wisconsin, Madison 6, Wisconsin

Walsh, Joseph Leonard, 1936 (1), Widener 474, Harvard University, Cambridge 38, Massachusetts

Webster, David Locke, 1923 (3), Physics Department, Room 385, Stanford University, Stanford, California

Weiss, Paul Alfred, 1947 (8), Department of Zoology, University of Chicago, Chicago 37, Illinois

Weisskopf, Victor Frederick, 1952 (3), Department of Physics, Massachusetts Institute of Technology, Cambridge 39, Massachusetts

Went, Frits Warmolt, 1947 (7), California Institute of Technology, Pasadena 4, California

Werkman, Chester Hamlin, 1946 (9), Department of Bacteriology, Iowa State College, Ames, Iowa

Wetmore, Alexander, 1945 (8), Smithsonian Institution, Washington 25, D. C.

Wever, Ernest Glen, 1940 (12), Princeton University, Princeton, New Jersey

Weyl, Claus Hugo Hermann, 1940 (1), The Institute for Advanced Study, Princeton, New Jersey

Wheeler, John Archibald, 1952 (3), Palmer Physical Laboratory, Princeton University, Princeton, New Jersey

Whipple, George Hoyt, 1929 (10), School of Medicine and Dentistry, University of Rochester, 260 Crittenden Boulevard, Rochester 7, New York

Whitehead, John Boswell, 1932 (4), Johns Hopkins University, Baltimore 18, Maryland

Whitney, Hassler, 1945 (1), The Institute for Advanced Study, Princeton, New Jersey

Whyburn, Gordon Thomas, 1951 (1), School of Mathematics, University of Virginia, Charlottesville, Virginia

Wiggers, Carl John, 1951 (9), Cleveland Clinic, 2020 East 93rd Street, Cleveland 6, Ohio

Wigner, Eugene Paul, 1945 (3), 8 Ober Road, Princeton, New Jersey

Williams, Howel, 1950 (6), Department of Geological Sciences, University of California, Berkeley 4, California

Williams, John Warren, 1952 (5), Department of Chemistry, University of Wisconsin, Madison 6, Wisconsin

Williams, Robert R., 1945 (5), 297 Summit Avenue, Summit, New Jersey

Williams, Roger John, 1946 (5), Biochemical Institute, University of Texas, Austin 12, Texas

Willier, Benjamin Harrison, 1945 (8), Department of Biology, Johns Hopkins University, Baltimore 18, Maryland

Wilson, Edgar Bright, Jr., 1947 (5), Department of Chemistry, Harvard University, 12 Oxford Street, Cambridge 38, Massachusetts

Wilson, Edwin Bidwell, 1919 (3), Harvard School of Public Health, 695 Huntington Avenue, Boston 15, Massachusetts

Wilson, Ralph Elmer, 1950 (2), 605 Acacia Avenue, Corona del Mar, California

Wilson, Robert Erastus, 1947 (4), 910 South Michigan Avenue, Chicago 80, Illinois

Wintersteiner, Oskar, 1950 (9), Squibb Institute for Medical Research, New Brunswick, New Jersey

Wislocki, George Bernays, 1941 (8), Harvard Medical School, 25 Shattuck Street, Boston 15, Massachusetts

Wolbach, Simeon Burt, 1938 (10), Division of Nutritional Research, The Children's Hospital, 300 Longwood Avenue, Boston 15, Massachusetts

Wolfrom, Melville Lawrence, 1950 (5), Department of Chemistry, Ohio State University, Columbus 10, Ohio

Wood, Harland Goff, 1953 (9), Department of Biochemistry, School of Medicine, Western Reserve University, Cleveland 6, Ohio

Wood, Robert Williams, 1912 (3), Johns Hopkins University, Baltimore 18, Maryland

Woodring, Wendell Phillips, 1946 (6), United States Geological Survey, Department of the Interior, Washington 25, D. C.

Woodward, Robert Burns, 1953 (5), Department of Chemistry, Harvard University, 12 Oxford Street, Cambridge 38, Massachusetts

Woodworth, Robert Sessions, 1921 (12), Columbia University, New York 27, New York

Woolley, Dilworth Wayne, 1952 (9), Rockefeller Institute for Medical Research, 66th Street and York Avenue, New York 21, New York

Wright, Frederick Eugene, 1923 (6), 2134 Wyoming Avenue, N. W., Washington 8, D. C.

Wright, Sewall Green, 1934 (8), Department of Zoology, University of Chicago, Chicago 37, Illinois

Wright, William Hammond, 1922 (2), 60 North Keeble Avenue, San Jose 26, California

Wulf, Oliver Reynolds, 1949 (13), United States Weather Bureau, Gates and Crellin Laboratories of Chemistry, California Institute of Technology, Pasadena 4, California

Wyckoff, Ralph Walter Graystone, 1949 (5), Laboratory of Physical Biology, National Institutes of Health, Bethesda 14, Maryland

Yerkes, Robert Mearns, 1923 (12), Yale University School of Medicine, 333 Cedar Street, New Haven 11, Connecticut

Yost, Don Merlin Lee, 1944 (5), California Institute of Technology, Pasadena 4, California

Young, William Gould, 1951 (5), Department of Chemistry, University of California, Los Angeles 24, California

Zachariasen, Frederik William Houlder, 1949 (3), Department of Physics, University of Chicago, Chicago 37, Illinois

Zariski, Oscar, 1944 (1), Department of Mathematics, Harvard University, Cambridge 38, Massachusetts

Zworykin, Vladimir Kosma, 1943 (4), Laboratories Division, Radio Corporation of America, Princeton, New Jersey

Number of Members July 1, 1953: 514.

MEMBERS EMERITI

Allen, Charles Elmer, 1924 (7), Biology Building, University of Wisconsin, Madison 6, Wisconsin

Anderson, John August, 1928 (2), P. O. Box 332, Pasadena 17, California

Benedict, Francis Gano, 1914 (9), Machiasport, Maine

Child, Charles Manning, 1935 (8), School of Biological Sciences, Jordan Hall, Stanford University, Stanford, California

Dickson, Leonard Eugene, 1913 (1), Route #2, Joliet, Illinois

Hulett, George Augustus, 1922 (5), 44 Washington Road, Princeton, New Jersey

Merrill, Elmer Drew, 1923 (7), Arnold Arboretum, Jamaica Plain, Massachusetts

Novy, Frederick George, 1924 (10), 721 Forest Avenue, Ann Arbor, Michigan

Palache, Charles, 1934 (6), Box 205-D, R. D. 3, Charlottesville, Virginia
Parker, George Howard, 1913 (8), Museum of Comparative Zoology at Harvard College, Oxford Street, Cambridge 38, Massachusetts
Stratton, George Malcolm, 1928 (10), University of California, Berkeley 4, California
Swanton, John Reed, 1932 (11), 22 George Street, Newton 58, Massachusetts
Vandiver, Harry Shultz, 1934 (1), Box 7881, University of Texas, Austin 12, Texas
Whitney, Willis Rodney, 1917 (5), General Electric Company, Schenectady 5, New York

Number of Members Emeriti July 1, 1953: 14.

FOREIGN ASSOCIATES

The number in parentheses following the year of election indicates association within the sections of the National Academy of Sciences.

Adrian, Edgar Douglas, 1941 (9), Trinity College, Cambridge, England
Alexandroff, Paul A., 1947 (1), Mathematical Institute of the Academy of Sciences of the U.S.S.R., Bolshaya Kalushskaya 19, Moscow, U.S.S.R.
Bailey, Sir Edward, 1944 (6), 19 Greenhill Gardens, Edinburgh 10, Scotland
Bartlett, Sir Frederic Charles, 1947 (12), University of Cambridge, The Psychological Laboratory, Downing Place, Cambridge, England
Best, Charles Herbert, 1950 (9), Banting and Best Department of Medical Research, University of Toronto, Toronto, Canada
Bjerrum, Niels, 1952 (5), The Royal Veterinary and Agricultural College, Copenhagen, Denmark
Bohr, Niels, 1925 (3), Institute for Theoretical Physics, Blegdamsvej 15, Copenhagen, Denmark
Bordet, Jules, 1935 (9), Pasteur Institute, Rue du Remorquer, 28, Brussels, Belgium
Bragg, Sir William Lawrence, 1945 (3), Cavendish Laboratory, The University, Cambridge, England
de Broglie, Prince Louis, 1948 (3), 94 Rue Perronet, Neuilly-sur-Seine, France
Caso, Alfonso, 1943 (11), Av. Sudderman No. 312, Col. Chapultepec-Morales, Mexico 5, D. F.
Chapman, Sydney, 1946 (1), The Geophysical Institute, College, Alaska, U.S.A.
Dale, Sir Henry Hallett, 1940 (9), The Wellcome Trust, 28 Portman Square, London, W.1, England

Dirac, Paul Adrien Maurice, 1949 (1), Department of Mathematics, St. John's College, Cambridge, England

Eskola, Pentti Eelis, 1951 (6), Helsinki University, Snellmanink. 5, Helsinki, Finland

Fisher, Ronald Aylmer, 1948 (8), Department of Genetics, University of Cambridge, 44 Storey's Way, Cambridge, England

von Frisch, Karl, 1951 (8), The Zoological Institute, University of Munich, Munich, Germany

Hadamard, Jacques, 1926 (1), 12 Rue Emile Faguet, Paris XIV, France

Helland-Hansen, Björn, 1947 (13), Chr. Michelsens Institutt for Videnscap, Bergen, Norway

Hill, Archibald Vivian, 1941 (9), 16 Bishopswood Road, Highgate, London, N.6, England

Hill, James Peter, 1940 (8), Kanimbla, Dollis Avenue, London N.3, England

Houssay, Bernardo Alberto, 1940 (9), Viamonte 2790, Buenos Aires, Argentina

Jeffreys, Harold, 1945 (13), St. John's College, Cambridge, England

Jones, Sir Harold Spencer, 1943 (2), Royal Greenwich Observatory, Herstmonceux Castle, Hailsham, Sussex, England

Kapitza, Peter Leonidovich, 1946 (3), Institute for Physical Problems, Academy of Sciences of the U.S.S.R., Moscow, U.S.S.R.

Karrer, Paul, 1945 (5), University of Zurich, Zurich, Switzerland

Keith, Sir Arthur, 1941 (11), Buckston Browne Farm, Downe, Farnborough, Kent, England

Kluyver, Albert Jan, 1950 (7), Technical University, Delft, The Netherlands

Levi, Giuseppe, 1940 (8), Instituto di Anatomia Umana, Corso Massimo D'Azeglio, 52, Turin, Italy

Lim, Robert K. S., 1942 (9), The Miles-Ames Research Laboratory, Elkhart, Indiana

Linderstrøm-Lang, Kaj Ulrik, 1947 (5), Chemical Division, Carlsberg Laboratory, Copenhagen, Denmark

Oort, Jan Hendrik, 1953 (1), Observatory of Leiden, Leiden, The Netherlands

Penfield, Wilder, 1953 (10), Montreal Neurological Institute, 3801 University Street, Montreal 2, Canada

Pieron, Henri, 1949 (12), Institute of Psychology, University of Paris, Paris, France

Reichstein, Tadeus, 1952 (5), Organisch-chemische Anstalt, St. Johanns-Ring 19, Basel, Switzerland

Robinson, Sir Robert, 1934 (5), Dyson Perrins Laboratory, South Parks Road, Oxford, England

Ruzicka, Leopold, 1944 (5), Department of Organic Chemistry, Institute of Technology, Zurich, Switzerland

Southwell, Sir Richard Vynne, 1943 (4), The Old House, Trumpington, Cambridge, England

Svedberg, The, 1945 (5), Fysikalisk-Kemiska Institutionen, University of Uppsala, Uppsala, Sweden

Sverdrup, Harald Ulrik, (1945)* 1952 (13), Norsk Polarinstitutt, Observatoriegt 1, Oslo, Norway

Taylor, Sir Geoffrey I., 1945 (1), Trinity College, Cambridge, England

Thomson, Sir Godfrey, 1951 (12), Edinburgh University, 5 Ravelston Dykes, Edinburgh 4, Scotland

Tiselius, Arne W. K., 1949 (9), Institute of Biochemistry, Uppsala University, Uppsala, Sweden

Vallee-Poussin, C. de la, 1929 (1), University of Louvain, Louvain, Belgium

Vening Meinesz, Felix Andries, 1939 (13), Potgieterlaan 5, Amersfoort, The Netherlands

Watson, D. M. S., 1938 (8), University College, Gower Street, London, W.C.1, England

Wieland, Heinrich, 1932 (5), Sophienstrasse 9, Munich 2 NW, Germany

Winge, Öjvind, 1949 (9), Department of Physiology, Carlsberg Laboratory, Copenhagen (Valby), Denmark

Yukawa, Hideki, 1949 (3), Yukawa Hall, Kyoto University, Kyoto, Japan

Number of Foreign Associates July 1, 1953: 49.

* Dr. Sverdrup was elected a member of the Academy in 1945. He resigned on April 2, 1951, on which date he had maintained residence in Norway for three years, thereby losing his American citizenship. He was elected a foreign associate on April 29, 1952.

SECTIONS

(1) *Mathematics*—28 members

McShane, E. J., *Chairman* (1955)
Ahlfors, L. V.
Albert, A. A.
Alexander, J. W.
Bell, E. T.
Bochner, S.
Chevalley, C.
Coble, A. B.
Douglas, Jesse
Eisenhart, L. P.
Evans, G. C.
Hille, Einar
Kasner, Edward
Lefschetz, Solomon
Mac Lane, Saunders
Moore, R. L.
Morse, Marston
Murnaghan, F. D.
Smith, Paul A.
Stone, M. H.
Thomas, T. Y.
Veblen, Oswald
von Neumann, John
Walsh, J. L.
Weyl, Hermann
Whitney, Hassler
Whyburn, G. T.
Zariski, O.

Foreign Associates

Alexandroff, P. A.
Chapman, Sydney
Dirac, P. A. M.
Hadamard, Jacques
Taylor, Sir Geoffrey
Vallee-Poussin, C. de la

(2) *Astronomy*—26 members

Struve, Otto, *Chairman* (1956)
Abbot, C. G.
Adams, W. S.
Babcock, H. D.
Bowen, I. S.
Brouwer, Dirk
Clemence, G. M.
Hubble, E. P.
Joy, A. H.
King, A. S.
Kuiper, G. P.
Mayall, N. U.
Menzel, D. H.
Merrill, P. W.
Mitchell, S. A.
Nicholson, S. B.
Ross, F. E.
Russell, H. N.
Seares, F. H.
Shapley, Harlow
Slipher, V. M.
Spitzer, Lyman, Jr.
Stebbins, Joel
Trumpler, R. J.
Wilson, Ralph E.
Wright, W. H.

Foreign Associates

Jones, Sir H. Spencer
Oort, Jan Hendrik

(3) *Physics*—74 members

Slater, J. C., *Chairman* (1954)
Allison, S. K.
Alvarez, L. W.
Anderson, C. D.
Bacher, R. F.
Bainbridge, K. T.
Beams, J. W.
Bethe, H. A.
Birge, R. T.
Bloch, Felix
Bradbury, N. E.
Breit, Gregory
Bridgman, P. W.
Brillouin, Leon
Brode, R. B.
Coblentz, W. W.
Compton, A. H.
Compton, K. T.
Condon, E. U.
Coolidge, W. D.
Davis, Bergen
Davisson, C. J.
Dennison, D. M.
DuBridge, L. A.
DuMond, J. W. M.
Dunning, J. R.
Einstein, Albert
Epstein, P. S.
Fermi, Enrico

Franck, James	McMillan, E. M.	Seitz, Frederick
Gamow, George	Millikan, R. A.	Serber, R.
Goudsmit, S. A.	Mulliken, R. S.	Stern, Otto
Houston, W. V.	Nier, A. O. C.	Stewart, G. W.
Hull, A. W.	Oppenheimer, J. R.	Street, J. C.
Ives, H. E.	Pegram, G. B.	Teller, Edward
Kemble, E. C.	Pierce, G. W.	Tuve, M. A.
Kent, R. H.	Purcell, E. M.	Van Vleck, J. H.
Kerst, D. W.	Rabi, I. I.	Webster, D. L.
Land, E. H.	Ramsey, N. F.	Weisskopf, V. F.
Lauritsen, C. C.	Robertson, H. P.	Wheeler, J. A.
Lawrence, E. O.	Rossi, Bruno	Wigner, E. P.
Loomis, F. W.	Saunders, F. A.	Wilson, Edwin B.
Lyman, Theodore	Schwinger, Julian	Wood, R. W.
Mason, Max	Segrè, E.	Zachariasen, W. H.

Foreign Associates

Bohr, Niels	de Broglie, Prince Louis	Kapitza, P. L.
Bragg, Sir Lawrence		Yukawa, Hideki

(4) *Engineering*—37 members

Terman, F. E., *Chairman* (1956)	Gibbs, W. F.	Savage, J. L.
	Gilliland, E. R.	Shockley, W.
Adams, C. A.	Greenewalt, C. H.	Slepian, Joseph
Briggs, L. J.	Hoover, Herbert	Soderberg, C. R.
Buckley, O. E.	Hunsaker, J. C.	Stratton, J. A.
Bush, Vannevar	Jeffries, Zay	Suits, C. G.
Cochrane, E. L.	Kelly, M. J.	Thomas, C. A.
Curme, G. O., Jr.	Kettering, C. F.	Timoshenko, Stephen
Den Hartog, J. P.	Lewis, W. K.	von Kármán, T.
Dryden, H. L.	Loomis, A. L.	Whitehead, J. B.
Durand, W. F.	Merica, P. D.	Wilson, Robert E.
Fletcher, Harvey	Murphree, E. V.	Zworykin, V. K.
Foote, P. D.	Raymond, A. E.	

Foreign Associate

Southwell, Sir Richard V.

(5) *Chemistry*—69 members

Kistiakowsky, G. B., *Chairman* (1956)	Badger, R. M.	Bolton, E. K.
	Bartlett, P. D.	Conant, J. B.
Adams, Roger	Bogert, M. T.	Cope, A. C.

265
2

Craig, L. C.
Daniels, Farrington
Debye, Peter
Edsall, J. T.
Elderfield, R. C.
Eyring, Henry
Fieser, L. F.
Flory, P. J.
Folkers, Karl
Fuoss, R. M.
Fuson, R. C.
Giauque, W. F.
Gilman, Henry
Hammett, L. P.
Harned, H. S.
Hendricks, S. B.
Hildebrand, J. H.
Hirschfelder, J. A.
Jacobs, W. A.
Johnson, J. R.
Johnson, W. S.
Keyes, F. G.
Kharasch, M. S.
Kirkwood, J. G.
Kraus, C. A.
La Mer, V. K.
Langmuir, Irving
Latimer, W. M.
Libby, W. F.
Lind, S. C.
Longsworth, L. G.
MacInnes, D. A.
Marvel, C. S.
Mayer, J. E.
McElvain, S. M.
Mees, C. E. K.
Niemann, C. G.
Noyes, W. A., Jr.
Onsager, Lars
Pauling, Linus
Pitzer, K. S.
Rodebush, W. H.
Rossini, F. D.
Scatchard, George
Schlesinger, H. I.
Seaborg, G. T.
Shedlovsky, Theodore
Small, L. F.
Smith, L. I.
Spedding, F. H.
Tishler, Max
Urey, H. C.
Williams, J. W.
Williams, Robert R.
Williams, Roger J.
Wilson, E. Bright, Jr.
Wolfrom, M. L.
Woodward, R. B.
Wyckoff, R. W.
Yost, D. M.
Young, W. G

Foreign Associates

Bjerrum, Niels
Karrer, Paul
Linderstrøm-Lang, K. U.
Reichstein, Tadeus
Robinson, Sir Robert
Ruzicka, Leopold
Svedberg, The
Wieland, Heinrich

(6) *Geology*—36 members

Buddington, A. F., *Chairman* (1954)
Allen, E. T.
Berkey, C. P.
Birch, Francis
Blackwelder, Eliot
Bowen, N. L.
Bradley, W. H.
Bucher, W. H.
Buerger, M. J.
Chaney, R. W.
Cloos, Ernst
Daly, R. A.
Day, A. L.
DeGolyer, E. L.
Dunbar, C. O.
Gilluly, James
Gregory, W. K.
Hess, H. H.
Hewett, D. F.
Kelley, W. P.
Knopf, Adolph
Larsen, E. S., Jr.
Leith, C. K.
Longwell, C. R.
Lovering, T. S.
Mead, W. J.
Mendenhall, W. C
Nolan, T. B.
Reeside, J. B., Jr.
Rubey, W. W.
Ruedemann, Rudolf
Schairer, J. F.
Simpson, G. G.
Williams, Howel
Woodring, W. P.
Wright, F. E.

Foreign Associates

Bailey, Sir Edward
Escola, Pentti

(7) *Botany*—42 members

Raper, K. B., *Chairman* (1956)
Babcock, E. B.
Bailey, I. W.
Bailey, L. H.
Barker, H. A.
Beadle, G. W.
Blakeslee, A. F.
Bonner, James
Brink, R. A.
Burkholder, P. R.
Chandler, W. H.
Clausen, R. E.
Cleland, R. E.
Couch, J. N.
Delbrück, Max
Dodge, B. O.
Duggar, B. M.
Emerson, Robert
Fred, E. B.
Goddard, D. R.
Horsfall, J. G.
Jones, D. F.
Kaufmann, B. P.
Kunkel, L. O.
Mangelsdorf, P. C.
McClintock, Barbara
Osterhout, W. J. V.
Rhoades, M. M.
Riker, A. J.
Robbins, W. J.
Sax, Karl
Sinnott, E. W.
Smith, Gilbert M.
Stadler, L. J.
Stakman, E. C.
Stebbins, G. L., Jr.
Thimann, K. V.
Thom, Charles
Van Niel, C. B.
Waksman, S. A.
Walker, J. C.
Went, F. W.

Foreign Associate

Kluyver, A. J.

(8) *Zoology and Anatomy*—46 members

Willier, B. H., *Chairman* (1955)
Allee, W. C.
Bartelmez, G. W.
Bigelow, H. B.
Bodine, J. H.
Castle, W. E.
Cleveland, L. R.
Corner, G. W.
Danforth, C. H.
Demerec, Milislav
Detwiler, S. R.
Dobzhansky, Th.
Dunn, L. C.
Goldschmidt, R. B.
Hamburger, Viktor
Harrison, R. G.
Hartman, C. G.
Harvey, E. N.
Herrick, C. J.
Hisaw, F. L.
Hubbs, C. L.
Hutchinson, G. E.
Irwin, M. R.
Jacobs, M. H.
Kellogg, Remington
Lewis, W. H.
Metz, C. W.
Moore, C. R.
Muller, H. J.
Nicholas, J. S.
Painter, T. S.
Patterson, J. T.
Riddle, Oscar
Romer, A. S.
Schmitt, F. O.
Schrader, Franz
Smith, Philip E.
Sonneborn, T. M.
Stern, Curt
Sturtevant, A. H.
Taliaferro, W. H.
Twitty, V. C.
Weiss, Paul
Wetmore, Alexander
Wislocki, G. B.
Wright, Sewall

Foreign Associates

Fisher, R. A.
von Frisch, Karl
Hill, J. P.
Levi, Giuseppe
Watson, D. M. S.

(9) *Physiology and Biochemistry*—60 members

Hastings, A. B., *Chairman* (1954)
Anderson, R. J.
Ball, E. G.
Bard, Philip
Bronk, D. W.
Carlson, A. J.
Carter, H. E.
Clark, W. M.
Clarke, H. T.
Cohn, E. J.
Cori, Carl F.
Cori, Gerty T.
Davis, Hallowell
Doisy, E. A.
DuBois, E. F.
du Vigneaud, Vincent
Elvehjem, C. A.
Erlanger, Joseph
Evans, H. M.
Fenn, W. O.
Forbes, Alexander
Fruton, J. S.
Gasser, H. S.
Hartline, H. K.
Kendall, E. C.
King, C. G.
Lewis, H. B.
Link, K. P.
Lipmann, Fritz
Lloyd, D. P. C.
Loeb, R. F.
Long, C. N. H.
Lorente de Nó, R.
Mann, F. C.
Marshall, E. K., Jr.
Maynard, L. A.
McCollum, E. V.
Meek, W. J.
Northrop, J. H.
Oncley, J. L.
Peters, J. P.
Richards, A. N.
Rittenberg, David
Rose, W. C.
Schmidt, C. F.
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CONTENTS

Page

BIOCHEMISTRY.—THE EFFECT OF ANTIBIOTICS ON UREA BREAKDOWN IN MICE . . . . . *By Renee Z. Dintzis and A. Baird Hastings* 571

BIOCHEMISTRY.—PATH OF ORNITHINE SYNTHESIS IN *Escherichia Coli* . . . . . *By Henry J. Vogel* 578

BOTANY.—PULCHERRIMIN, THE PIGMENT OF *Candida Pulcherrima* . . . . . *By A. J. Kluyver, J. P. van der Walt, and A. J. van Triet* 583

BOTANY.—TAXONOMIC SIGNIFICANCE OF ROOT-HAIR DEVELOPMENT IN THE GRAMINEAE . . . . . *By John R. Reader and Kraft von Maltzahn* 593

CHEMISTRY.—THE MOLECULAR WEIGHT OF SOME LONG CHAIN SALTS IN BENZENE BY THE CRYOSCOPIC METHOD . . *By Charles H. Keith and Charles A. Kraus* 598

CHEMISTRY.—ACTION OF BORON TRIFLUORIDE ETHERATE ON β-CAROTENE . . . . . *By L. Wallcave, J. Leemann, and L. Zechmeister* 604

GENETICS.—MENDELIAN AND NON-MENDELIAN FACTORS AFFECTING THE CYTOCHROME SYSTEM IN *Neurospora Crassa* . . . . . *By M. B. Mitchell, H. K. Mitchell, and A. Tissières* 606

GENETICS.—INDUCTION OF CHROMOSOME BREAKAGE AT MEIOSIS BY A MAGNESIUM DEFICIENCY IN *Tradescantia* . . . . . *By Dale Steffensen* 613

GENETICS.—UNITY OF THE VEGETATIVE POOL IN PHAGE-INFECTED BACTERIA . . . . . *By N. Visconti and A. Garen* 620

GENETICS.—INDUCTION OF MUTATIONS IN A BACTERIAL VIRUS. . *By J. J. Weigle* 628

MATHEMATICS.—RELATIONS ON ITERATED REDUCED POWERS . . *By José Adem* 636

MATHEMATICS.—CONSTRAINED GAMES AND LINEAR PROGRAMMING . . . . . *By A. Charnes* 639

MATHEMATICS.—ON ARITHMETIC GENERA OF ALGEBRAIC VARIETIES . . . . . *By K. Kodaira and D. C. Spencer* 641

MATHEMATICS.—EXTENSION THEOREMS FOR SOLUTIONS OF IRREFLEXIVE RELATIONS. . . . . *By Moses Richardson* 649

MATHEMATICS.—A FIRST APPROXIMATION TO HOMOTOPY THEORY . . . . . *By E. H. Spanier and J. H. C. Whitehead* 655

MATHEMATICS.—COHOMOLOGY AND THE RIEMANN-ROCH THEOREM. . . . . *By D. C. Spencer* 660

PHYSICS.—IMAGE THEORY OF SUPERPOSED VECTOR SETS . . . *By M. J. Buerger* 669

PHYSICS.—SOLUTION FUNCTIONS FOR SOLVING SUPERPOSED PATTERSON SYNTHESES . . . . . *By M. J. Buerger* 674

PHYSICS.—AN INTERSECTION FUNCTION AND ITS RELATIONS TO THE MINIMUM FUNCTION OF X-RAY CRYSTALLOGRAPHY . . . . . *By M. J. Buerger* 678

PHYSICS.—ABOUT SPACE-TIME ASYMMETRY IN THE REALM OF CLASSICAL GENERAL AND CRYSTAL PHYSICS . . . . . *By H. Zocher and C. Török* 681

ZOOLOGY.—ACTION POTENTIALS FROM AN ARTHROPOD OCELLUS: THE MEDIAN EYE OF LIMULUS. . . . . *By Talbot H. Waterman* 687

MATHEMATICS.—AN ACKNOWLEDGMENT . . . . . *By H. F. Weinberger* 694